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Pest Risk Analysis for

Amaranthus tuberculatus

Amaranthus tuberculatus in soybean. Bob Hartzler (EPPO Global database)

September 2020

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The risk assessment follows EPPO standard PM 5/5(1) *Decision-Support Scheme for an Express Pest Risk Analysis* (available at http://archives.eppo.int/EPPOStandards/pra.htm), as recommended by the Panel on Phytosanitary Measures. Pest risk management (detailed in appendix 1) was conducted according to the EPPO Decision-support scheme for quarantine pests PM 5/3(5). The risk assessment uses the terminology defined in ISPM 5 *Glossary of Phytosanitary Terms* (available at https://www.ippc.int/index.php).

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Based on this PRA, *Amaranthus tuberculatus* was added to the EPPO A2 List. Measures for grains of *Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*; Seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*; seed mixtures and native seeds; as well as used agricultural machinery and equipment are recommended.

Pest Risk Analysis for

Amaranthus tuberculatus (Moq) J.D. Sauer (Amaranthaceae)

PRA area: EPPO region
Prepared by: EWG on *Amaranthus palmeri* and *A. tuberculatus*

Date: 24-28 February 2020
Further reviewed and amended by EPPO core members. and Panel on Invasive Alien Plants.

Composition of the Expert Working Group (EWG)

BRUNDU Giuseppe (Mr)	University of Sassari, Department of Agriculture, Italy
CHAPMAN Daniel (Mr)	University of Stirling, United Kingdom
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The first draft of the PRA was prepared by Swen Follak.

Ratings of likelihoods and levels of uncertainties were made during the meeting. These ratings are based on evidence provided in the PRA and on discussions in the group. Each EWG member provided a rating and a level of uncertainty anonymously and proposals were then discussed together in order to reach a final decision.

Following the EWG, the PRA was further reviewed by Ms Grousset F (EPPO) and the following core member: Gachet E.

The PRA, in particular the section on risk management, was reviewed and amended by the EPPO Panel on Invasive Alien Plants on 2020-06. The EPPO Working Party on Phytosanitary Regulations and Council agreed that *Amaranthus tuberculatus* should be added to the A2 List of pests recommended for regulation as quarantine pests in 2020.

Contents

Summary of the Express Pest Risk Analysis for *Amaranthus tuberculatus*
PRA area: EPPO region in 2020 (Albania, Algeria, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Guernsey, Hungary, Ireland, Israel, Italy, Jersey, Jordan, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, The Republic of North Macedonia, Malta, Moldova, Montenegro, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tunisia, Turkey, Ukraine, United Kingdom, Uzbekistan)
Describe the endangered area: The EWG considered that the endangered area includes agricultural environments situated to the North and east of the Mediterranean sea, especially in the agricultural production areas in Spain and Portugal, South of France, Italy, Adriatic coast (incl. Croatia), as well as the Pannonian Basin and countries bordering the Black Sea and central Asia (appendix 3, Fig. 5). The EWG considered the species distribution modelling conducted as part of this PRA (see Appendix 3) to be a realistic projection of the potential occurrence of *A. tuberculatus* in the EPPO region.
Main conclusions ***Amaranthus tuberculatus* presents a high phytosanitary risk for the endangered area with low uncertainty.** The likelihood of new introductions via bird feed is very high with a high uncertainty. The likelihood of new introductions to the EPPO region occurring via grain of soybean (*Glycine max*), haricot bean (*Phaseolus vulgaris*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*) is high with a moderate uncertainty. For seeds of beetroot (*Beta vulgaris*), *G. max*, cotton (*Gossypium hirsutum*), alfalfa (*Medicago sativa*), *P. vulgaris*, *S. bicolor* and *Z. mays*, the likelihood of new introductions is moderate with moderate uncertainty. Entry into the EPPO region via seed mixtures and native seeds is moderate with a high uncertainty. Within the EPPO region, currently the species mostly grows in ruderal habitats and along river systems, and to a lesser extent in agricultural environments. *A. tuberculatus* is capable of invading many summer crops in particular late sowing crops like maize and soybean. The high frequency of maize and soybean in the crop rotation system in many EPPO countries is a factor that may facilitate the establishment of *A. tuberculatus* once the field has become contaminated. The likelihood of further establishment outdoors is very high with a low uncertainty. Establishment in protected conditions is medium with a high uncertainty. Protected conditions, such as in nurseries and polytunnels, may offer appropriate conditions for the development of the pest. The potential for spread within the EPPO region is very high with a moderate uncertainty. *A. tuberculatus* can spread both naturally and via human-assisted spread. Seeds of *A. tuberculatus* can be moved through agricultural machinery and products (e.g. grains, seeds) within the EPPO region. The impacts of *A. tuberculatus* in North America are primarily the reduction of crop yields and increased management costs. The EWG considered the potential socio-economic impacts in the EPPO region will be high with a moderate uncertainty. *A. tuberculatus* is difficult to manage because of the species ability to produce large volumes of seeds and build up a persistant seed bank. This species has already been shown to easily develop resistance to various herbicide mode of actions in North America. The EWG considered that early detection and rapid responses are critical to avoid further spread and impact of *A. tuberculatus*.

Phytosanitary risk for the <u>*endangered area*</u> (*Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document***)**	High X	Moderate ☐	Low ☐
Level of uncertainty of assessment (see Section 17 for the justification of the rating. Individual ratings of uncertainty of entry, establishment, spread and impact are provided in the document)	High ☐	Moderate ☐	Low X

The EWG conducted two PRAs simultaneously on *A. tuberculatus* and *A. palmeri*. Text written in these PRAs have similarities. *Amaranthus tuberculatus* and *A. palmeri* are very similar in their biology and pathways, and both are important weeds in North America. However, these species show differences in terms of competitiveness and area of potential establishment in the EPPO region.

Other recommendations:

- perform a proper botanical survey in the EPPO region (e.g. during August when the inflorescence is visible). This can be performed for *A. palmeri* and *A. tuberculatus* together. If performed on the endangered area identified for *A. tuberculatus*, this would also cover the *A. palmeri* endangered area.
- take samples where *A. tuberculatus* is present to check herbicide resistance of the established populations.
- develop educational materials to help people identifying this species and promote early detection in new areas.

EPPO Pest Risk Analysis:

Amaranthus tuberculatus (Moq.) J.D.Sauer

Stage 1. Initiation

1.1 Reason for performing the PRA:

This PRA was conducted to determine the likelihood and extent of entry into and spread within the EPPO region of *A. tuberculatus*, along with the magnitude of its impacts. . In the United States (US), the species is considered to be one of the most problematic weeds in the Corn Belt (Midwestern US) (Sarangi *et al.*, 2019). The species has many weedy traits including high seed production, an extended emergence pattern and high growth rates that makes it highly competitive and harmful to crops and difficult to control (Costea *et al.*, 2005; Schryver *et al.*, 2017). As occurrences were often associated with soybeans and cereals in port areas (e.g. Sánchez Gullón & Verloove, 2013), the species was presumably introduced into the EPPO region as a contaminant of imported seed or grain for animal feed, and of products intended for use in the food industry. Thus, in addition to potential production and biodiversity, *A. tuberculatus* could negatively affect international trade and exchanges. At present, transient and established occurrences of the species are known from a number of EPPO countries, mainly on ruderal sites and along riverbanks, and to a lesser extent in crop fields. In Italy, alongside the river Po, *A. tuberculatus* has invaded native riparian herbaceous habitats (Iamonico, 2015a; CABI, 2020). The Panel on Invasive Alien Plants identified A. *tuberculatus* for risk assessment in 2019.

PRA area: EPPO region in 2020 (Albania, Algeria, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Guernsey, Hungary, Ireland, Israel, Italy, Jersey, Jordan, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, The Republic of North Macedonia, Malta, Moldova, Montenegro, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tunisia, Turkey, Ukraine, United Kingdom, Uzbekistan).

(see https://www.eppo.int/ABOUT_EPPO/eppo_members)

Stage 2. Pest risk assessment

1. Taxonomy: Kingdom: Plantae, Class *Angiospermae*, Order *Caryophyllales*, Family *Amaranthaceae*, Genus *Amaranthus*, Species *Amaranthus tuberculatus* (Moq) J.D.Sauer, Madroño 13: 18 (1955).

There were some nomenclatural and taxonomic complications with the species (Pratt & Clark, 2001; Costea *et al*., 2005). Sauer (1955) recognized two distinct species: *Amaranthus rudis* (distribution from the Great Plains west of the Mississippi, and from Texas to Iowa) and *Amaranthus tuberculatus* (within a more northern range, i.e. north of Missouri and Tennessee to the Great Lakes, Indiana to Ohio) based primarily on morphological differences (flower, fruits). Later, Pratt & Clark (2001) included *A. rudis* in synonymy of *A. tuberculatus*. They argued that any differentiation between the two related taxa was erased by agriculture and human-induced introduction and invasion. They recognized one polymorphic species – *A. tuberculatus*. Costea & Tardiff (2003) supported their conclusion of a single species but recognized two distinct taxa at the infraspecific level. The first one is *Amaranthus tuberculatus* var. *tuberculatus* (tall waterhemp), and the other one is *A. tuberculatus* var. *rudis* (common waterhemp). Costea *et al*. (2005) stated that *A. tuberculatus* var. *rudis* is more troublesome than *Amaranthus tuberculatus* var. *tuberculatus*. At present, the view of Pratt & Clark (2001) is generally accepted (Mosyakin & Robertson, 2003; Iamonico, 2015a; Iamonico, 2015b), i.e. that *A. rudis* is synonym of *A. tuberculatus*, and followed in this PRA.

Note: In the PRA, published information pertaining *A. rudis* or the two varieties *A. tuberculatus* var. *rudis* and *A. tuberculatus* var. *tuberculatus* are summarized below as *A. tuberculatus*.

EPPO code: AMATU

Synonyms
Acnida tuberculata Moquin-Tandon, *Acnida altissima* Riddell ex Moquin-Tandon, *Acnida altissima* var. *prostrata* (Uline & W. L. Bray) Fernald; *Acnida altissima* var. *subnuda* (S. Watson) Fernald, *Acnida concatenata* (Moquin-Tandon) Small; *Acnida subnuda* (S. Watson) Standley, *Acnida tamariscina* (Nuttall) Alph. Wood, *Acnida tamariscina* var. *concatenata* (Moquin-Tandon) Uline & W.L.Bray; *Acnida tamariscina* var. *tuberculata* (Moquin-Tandon) Uline & W.L.Bray; *Amaranthus ambigens* Standley; *Amaranthus rudis* J.D.Sauer

Ref: Mosyakin & Robertson (2003)

Common names:
English: rough-fruited water-hemp, tall water-hemp, rough-fruit amaranth, common waterhemp, Czech: laskavec tamaryškov, French: amarante rugueuse, acnide tuberculée, Hebrew: yarbuz haggadot, Italian: amaranto tuberculato, Russian: щирица бугорчатая. Dutch; oeveramarant

Plant type: Annual herbaceous.

Related species in the EPPO region:
The genus *Amaranthus* has a global distribution and comprises approximately 70 species (Iamonico, 2015a). Some 40 species are native to the Americas, and the remaining are native to Australia, Africa, Asia and Europe (Costea *et al.*, 2001). Examples of native *Amaranthus* species are listed below. Several non-native *Amaranthus* species occur in the EPPO region (see below) and here the list is not intended to be exhaustive but gives examples of species.

Examples of native species in the EPPO region:
Amaranthus blitum subsp. *blitum*, *A. graecizans* subsp. *sylvestris*. *Amaranthus* × *cacciatoi* and *A. hybridus* var. *bouchionii* listed by Iamonico (2015) as 'probably native' to Europe should be considered as neonative species sensu Stace & Crawley (2015).

Examples of non-native species in the EPPO region:
Amaranthus acutilobus, *A. albus*, *A. blitoides*, *A. caudatus*, *A. crispus*, *A. cruentus*, *A. deflexus*, *A. emarginatus subsp*. *emarginatus* var. *emarginatus*, *A. emarginatus* subsp. *emarginatus* var. *pseudogracilis*, *A. graecizans* subsp. *graecizans*, *A. hybridus* (excluding *A. hybridus* var. *bouchionii*), *A. hypochondriacus*, *A. muricatus*, *A. palmeri*, *A. polygonoides*, *A. powellii*, *A. retroflexus*, *A. spinosus*, *A. tamariscinus*, *A. tricolor*, *A. viridis* (Iamonico, 2015)

2. Pest overview

2.1 Introduction

Amaranthus tuberculatus is a small-seeded, summer annual species native to North America (Sauer, 1955). The species has become a major weed of agricultural fields and other disturbed habitats and it has been introduced in parts of North America far outside its original range (Costea *et al.*, 2005; Sarangi & Jhala, 2018). The species has several weedy attributes (e.g. ecological plasticity, rapid growth, prolific seed production). Nonetheless, in North America, the increase in the frequency and severity of infestations of *A. tuberculatus* has been predominately caused by changes in cultural practices (implementation of reduced and no-tillage systems) entirely reliant on herbicides for weed control and by the rapid development of herbicide-resistant biotypes (Costea *et al.*, 2005; Schryver *et al.*, 2017). *A. tuberculatus* was introduced into the EPPO region presumably in the middle of the 20th century.

2.2 Identification

Morphological identification

Misidentification of *Amaranthus* species can occur throughout its range due to the morphological variation within species and hybridization between species (Wetzel *et al.*, 1999). There are several identification keys that can be used to distinguish between *Amaranthus* species (e.g. Pratt & Clark, 2001; Horak *et al.*, 2019). Some of the key characteristics include flower morphology (needing magnification due to their small size), leaf shape, presence or absence of hair on the stem, seed head shape and seedling shape (Pratt & Clark, 2001). Iamonico (2015a) provides short descriptions of *Amaranthus* species that can been found in the EPPO region.

The following description is primarily based on Costea *et al.* (2005) and Mosyakin & Robertson (2003): *A. tuberculatus* is an annual herbaceous dioecious species, with a taproot, and it reproduces only by seeds. Stems of mature plants are erect, sometimes ascending, up to (5–) 20–200 (–300) cm in height, glabrous or with sparse hairs. Leaves are long petioled, ovate, rhombic-oblong to lanceolate-oblong (2–10 cm long, 1–3 cm wide), while the upper leaves are reduced and narrow. Male and female flowers occur on separate plants (dioecious) and the terminal inflorescences are 10–20 cm long, usually unbranched or with numerous panicled branches. Fruits are about 1.5 mm long, transversal (circumscissile) dehiscent at the middle, rugose, often reddish. Seeds are elliptic to obovate, dark reddish brown to dark brown, and 0.7-1 mm in diameter. *A. tuberculatus* seeds are among the smallest in the *Amaranthus* genus.

Appendix 2 includes images of the plant.

Molecular identification

Molecular methods are available to identify species within the genus using both plant material and seeds. A PCR test method has been developed to distinguish seven weedy *Amaranthus* species (incl. *A. tuberculatus*, as well as *A. palmeri*, *A. spinosus*, *A. retroflexus*, *A. blitoides*, *A. viridis*, and *A. hybridus*) from plant material based on intron 1 sequences from the 5-enolpyrvylshikimate-3-phosphate synthase gene (Wright *et al.*, 2015). Other methods are described in the literature (e.g. Wetzel *et al.*, 1999).

2.3 Hybridization

Amaranthus tuberculatus is a diploid taxon with a chromosome number of 2n = 32 (Trucco *et al.* 2006). The species can hybridize with other members of the subgenus *Acnida* and even with monoecious species belonging to subgenus *Amaranthus* (Costea *et al.*, 2005; Trucco *et al.*, 2006). *A. tuberculatus* has been shown by field and greenhouse experiments to be capable of hybridizing with *A. palmeri* (Gaines *et al.*, 2012). Hybridization between *A. palmeri* and *A. tuberculatus* occurred with frequencies in the field studies from < 0.2%. Hybridization occurs also between *A. tuberculatus* and *A. hybridus*, although genetic introgression between these species occurs only in one direction that is from *A. hybridus* to *A. tuberculatus* (Trucco *et al.* 2009). Interspecific hybridization was experimentally documented under field conditions for these two species (Trucco *et al.* 2005a, Trucco *et al.* 2005b).

2.4 Life cycle Seed germination and emergence of seedlings

In its native range, *A. tuberculatus* is a late emerging weed species. In southern Ontario (Canada), it typically initiates emergence from the beginning of June to August. In Iowa (USA), emergence begins in mid-May to late May and continues through early August. Flowering depends on the photoperiod.

2.4.1 Growth and reproduction

Plants grown under short-day conditions (8 h) require 14–16 days to initiate flowering, whereas plants grown under long-day conditions (16 h) need approximately 45 days. Flowering and seed set continue until the first frost (Costea *et al.*, 2005). *Amaranthus tuberculatus* is a dioecious *Amaranthus* species. Male flowers produce copious amounts of wind-dispersed pollen that can disperse over long distances (e.g. 300-800m) (Costea *et al.*, 2005; Liu *et al.*, 2012).

2.4.2 Seed production

Amaranthus tuberculatus is a prolific seed producer (Costea *et al.*, 2005; Heneghan & Johnson, 2017). When allowed to develop for a full growing season, *A. tuberculatus* has demonstrated the ability to produce up to 1 million seeds per plant (Steckel *et al.*, 2003). In cultivated fields, seed production declines as time of emergence is delayed because individuals accumulate less biomass due to competition with the previously established crop (Steckel *et al.*, 2003; Hartzler *et al.*, 2004; Nordby & Hartzler, 2004). For example, individuals emerging together with soybean (Iowa/USA) produced on average 300 000 seeds/plant while those emerging 50 d after planting produced only 3000 seeds/plant (Hartzler *et al.*, 2004). Mean seed yields were higher from the May (926,629 seeds/plant) and June (828,905 seeds/plant) crop establishment dates compared with the July (276,258 seeds/plant) crop establishment in a common garden experiment (Heneghan & Johnson, 2017). Viable seed production can occur as early as 7 d after pollination (Bell & Tranel, 2010).

2.4.3 Seed bank maintained in soil

Seeds persist for approximately 4 to 5 years in the soil in normal conditions (Buhler & Hartzler, 2001; Steckel *et al.*, 2007). Buhler & Hartzler (2001) found that 11% of seeds maintained their viability after 4 years of burial in the upper 5 cm of soil in Iowa/USA. The percentage of viable seeds recovered after 36 months on the soil surface was 4.3% compared with 5.3% at a 15-cm depth (multiple locations across the USA; Korres *et al.*, 2018). However, seeds buried at 20 cm soil depth retained 3% viability after 17 years (Nebraska/USA, Burnside *et al.*, 1996). The seed bank of *A. tuberculatus* in crop fields may contain tens of thousands of seeds per m^2 as shown by Buhler *et al.* (2001), comprising up to 90% of the total seed bank (Iowa/USA). *Amaranthus tuberculatus* has a rapid growth rate at an average of 0.135 cm of growth per growing degree day (Steckel, 2007).

2.4.4 Seed dispersal capacity

There is no specific information regarding natural seed dispersal of *A. tuberculatus* (Costea *et al.*, 2005). As for other *Amaranthus* species, seeds are considered to be dispersed by barochory (falling from the parent plant) and hydrochory (dispersal via water) (Costea *et al.*, 2004). Possible dispersal mechanisms are detailed in section 11.

2.5 Habitats

In its native range, *A. tuberculatus* was initially described as growing in wet areas such as margins of rivers, ponds, marshes, lakes, and creeks (Sauer, 1955). Nowadays, it is found in any disturbed habitats lacking permanent vegetation, in particular in crop fields, along roadsides, and railroads up to 1000 m above sea level (Sauer, 1955; Mosyakin & Robertson 2003, Costea *et al.* 2005; see section 2.6).

Within the EPPO region, *A. tuberculatus* is present in ruderal habitats including port areas and naturally disturbed habitats like riparian systems, and to a lesser extent crop fields (Iamonico, 2015a; Iamonico, 2015b; Verloove, 2019).

See section 7 for further details on habitats in the EPPO region.

2.6 Association with crops

As a summer annual species, *A. tuberculatus* is able to persist and thrive in crops which have a similar lifecyle to the species. In the USA, the main crops *A. tuberculatus* is associated with include fields of crops such as cereals (*Sorghum bicolor*, *Zea mays*), cotton (*Gossypium hirsutum)*, maize (*Zea mays*), soybean

(*Glycine max*), beet (*Beta vulgaris*). It can also become a problem in fields of *Phaesolus vulgaris* and *Medicago sativa*.

Table 1. Main crops which *Amaranthus tuberculatus* is associated with in North America. Country codes are based on ISO Country codes (US: USA; CA: Canada)

Crop	Country	Reference
Zea mays	US, CA	Costea *et al*., 2005
Glycine max	US, CA	Costea *et al*., 2005
Beta vulgaris	US	Peters *et al*., 2019
Phaseolus vulgaris	US	Hartzler pers. comm., 2020
Medicago sativa	US	Hartzler pers. comm., 2020
Sorghum bicolor	US	Grichar, 2006
Gossypium hirsutum	US	Werner *et al*., 2019

A. tuberculatus may be associated with other summer crops in its area of origin.

2.7 Environmental requirements

Amaranthus tuberculatus occurs over a wide climatic range. In North America, *A. tuberculatus* occurs preferably at latitudes between 45° and 30° North (USDA-NRCS, 2019). Costea *et al*. (2005) summarizes the ecological preferences of this species as follows: "thermophyte, hygrophyte to mesophyte, heliophyte and nitrophilous". It can tolerate a broad range of soil types and textures but prefers those that are well-drained and rich in nutrients (Costea *et al.*, 2005). *A. tuberculatus* also grows well on poorly drained soils (CABI, 2020). Plants can survive temporary flooding but have no salinity or frost tolerance (Costea *et al.*, 2005).

The species can survive and reproduce even under a high degree and duration of water stress (low water conditions) according to Sarangi *et al.* (2016). Grantz *et al.* (2019) showed that *A. tuberculatus* (Californian biotype) was highly tolerant to ozone and deficit irrigation (33% of field capacity) under greenhouse conditions. This competitive advantage allows the species to occur in areas that are prone to drought, such as recently discovered in southern and central California (Calflora, 2019), and facilitate its weedy behaviour there.

The species requires warm temperatures for germination and growth. Steckel *et al.* (2004) observed highest germination rates under a temperature range between 25 and 35 °C under controlled conditions (seeds collected from Illinois (USA)). Above 20 °C, the species had higher germination rates with an alternating temperature regimen (= temperature varied 40 % of each constant temperature in a sinusoidal fashion during a 14-d period) than to the constant regimen (= constant temperature during a 14-d period). Seeds failed to germinate significantly when exposed to temperatures less than 20 °C. The minimum temperature for germination was 10°C for populations from Iowa (USA) and over 15/10 °C for populations from Kansas (USA) (Guo & Al-Khatib, 2003; Leon *et al.*, 2004).

Growth of *A. tuberculatus* is influenced by both temperature and light. For example, biomass accumulation, height and root volume were higher at 25/20 °C and 35/30 °C than at 15/10°C according to a greenhouse trial by Guo and Al-Khatib (2003). Steckel *et al.* (2003) demonstrated that in full sunlight a *A. tuberculatus* plant produced 720 g biomass and under 40 and 68 % shading plants produced only 550 and 370 g, respectively (under field conditions, Illinois/USA).

3. Is the pest a vector?	**Yes**	☐	**No**	**X**
4. Is a vector needed for pest entry or spread?	**Yes**	☐	**No**	**X**

5. Regulatory status of the pest

In the USA, Wisconsin law prohibits the sale of agricultural seed containing *A. tuberculatus* seed (USDA, 2019a; https://www.ams.usda.gov/rules-regulations/fsa).

In Canada, *A. tuberculatus* is listed as a “Primary Noxious Weed Seeds” under the Weed Seeds Order of the Seeds Act (http://www.gazette.gc.ca/rp-pr/p2/2016/2016-05-18/html/sor-dors93-eng.html).

In Argentina, *A. tuberculatus* is included on the A1 (absent) List at 2019 (EPPO, 2020a).

In Australia, *A. tuberculatus* (listed as *A. rudis*) is listed as a quarantine pest.

A risk assessment has been produced by the Chinese Inspection and Quarantine Bureau (Han *et al.*, 2013). The genus *Amaranthus* is regulated in China.

6. Distribution

Amaranthus tuberculatus is native to North America where the species is recorded as being weedy in the United States and Canada (USDA-NRCS, 2019; Costea *et al.*, 2005). Its native range is Central and Eastern Central USA. The species “… has gone from virtual obscurity to being the most commonly encountered and troublesome weed” in agriculture, in particular in the Midwestern United States over the last 30 year (Sarangi & Jhala, 2018; Sarangi *et al*., 2019). In North America, *A. tuberculatus* occurs mostly at latitudes between 45° and 30° North (USDA-NRCS, 2019).

Amaranthus tuberculatus was introduced into the EPPO region presumably in the middle of the 20th century. However, the species might have already been introduced before (see below Switzerland). The early records were of small and transient populations scattered across the EPPO region (e.g. Austria and the United Kingdom). The first naturalized populations presumably occurred from the middle of 1970s onwards in Italy.

Region	Distribution	Status	References and comments
America	Ontario	Introduced/ native	Mosyakin & Robertson (2003), VASCAN (2019), Schryver *et al.* (2017), Costea *et al.* (2005)
Canada	Manitoba	Introduced	VASCAN (2019), Mosyakin & Robertson (2003)
	British Columbia	Introduced	Transient (Costea *et al.* 2005)
	Prince Edward Island	Introduced	Mosyakin & Robertson (2003); reported from the region, but not established or erroneously determined according to VASCAN (2019)
	Quebec	Introduced/ native	VASCAN (2019), Mosyakin & Robertson (2003), Schryver *et al.* (2017), Costea *et al.* (2005)
USA	Alabama	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Arkansas	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	California	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Colorado	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Connecticut	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Delaware	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Georgia	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Idaho	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Illinois	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Indiana	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Iowa	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Kansas	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Kentucky	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Louisiana	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Maine	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Maryland	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Massachusetts	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Michigan	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Minnesota	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Mississippi	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Missouri	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Nebraska	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Nevada	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	New Hampshire	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	New Mexico	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	New York	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)

	North Carolina	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	North Dakota	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Ohio	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Oklahoma	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Pennsylvania	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	South Carolina	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	South Dakota	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Tennessee	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Texas	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Vermont	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Washington	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	West Virginia	Native	Mosyakin & Robertson (2003); Plants of the World Online (2019)
	Wisconsin	Introduced	Mosyakin & Robertson (2003); Plants of the World Online (2019)
EPPO region	Austria	Introduced	Absent: no longer present (Melzer, 1954; Melzer, 1957; Follak per. communication, 2019)
	Belgium	Introduced	Transient (Verloove, 2019)
	Bosnia and Herzegovina	Introduced	Transient (Maslo et al., 2020)
	Czech Republic	Introduced	Transient (Pyšek *et al.*, 2012)
	Denmark	Introduced	Transient (as *A. rudis*. Jonsell, 2001).
	Finland	Introduced	Transient (FinBIF, 2019)
	Germany	Introduced	Transient (Buttler *et al.* 2018), but probably establishing around Mannheim (Junghans, 2016)
	Israel	Introduced	Locally established (Danin & Fragman-Sapir, 2019; Cafri, pers. comm., 2019)
	Italy	Introduced	Established. Lombardy, Emilia-Romagna, Veneto and Marche (invasive); Piemonte, Friuli Venezia Giulia, Tuscany Trentino-Alto Adige (locally established, transient) (Iamonico, 2015a; Iamonico 2015b; Galasso *et al.*, 2018; Acta Plantarum, 2019)
	Jordan	Introduced	Transient (Taifour *et al.*, 2016)
	Netherlands	Introduced	Present, probably establishing (NDFF & FLORON, 2019; Duistermaat, pers. comm., 2019)
	Romania	Introduced	Present, probably establishing (Anastasiu *et al.*, 2011; Memedemin *et al.*, 2016)
	Russian Federation	Introduced	Transient (Czerepanov, 1995)
	Spain	Introduced	Present, probably establishing (Sánchez Gullón & Verloove, 2013)
	Switzerland	Introduced	Absent (Buholzer, pers. comm., 2019)
	United Kingdom	Introduced	Absent no longer present (Brenan 1961; BRC, 2019)
	Ukraine	Introduced	Transient (Mosyakin & Fedoronchuk, 1999; Yavorska, 2009)

*The transient status may not reflect the initial wording of the referred publication and is used following the IPPC definition (transience: Presence of a pest that is not expected to lead to establishment [ISPM 8, 1998] (ISPM 5, 2019)).

Specific details about the distribution in selected EPPO countries (where available)

Austria: *Amaranthus tuberculatus* specimens were found in Styria in the 1950s along railway tracks in Graz (Melzer, 1954; Melzer, 1957). At present, no occurrence of the species is known, and the species is absent (Follak pers. comm., 2019).

Belgium: *Amaranthus tuberculatus* is a "regular but ephemeral alien" since 1983 according to Verloove (2019). It has been found as a soybean alien in port areas (mainly in Antwerpen and Gent). It has also been recorded in 2003 along the river Maas near Eelen (Verloove, 2019).

Czech Republic: Transient occurrences have been documented (as *A. rudis*). Its first occurrence is from 1967 (Pyšek *et al.*, 2012).

Netherlands: *Amaranthus tuberculatus* has been recorded along the rivers Maas and Waal (van der Meijden *et al.*, 2003; van der Meijden & Holverda, 2006; NDFF & FLORON, 2019). It can be found on the banks of streams, in nutrient-rich marshes and on mud flats. It has also been recorded in damp places in fields and meadows, sand storage places and ruderal places. The species is probably establishing (Duistermaat, pers. comm., 2019).

Italy: The species is distributed in Northern Italy (Iamonico, 2015a; Iamonico 2015b; Galasso *et al.*, 2018; Acta Plantarum, 2019). Early records of *A. tuberculatus* are from the mid-1970s along the Po River near Cava Manara/Lombardy (1975) (Soldano 1982) and Castelnuovo/ Bocca d'Adda (1974, "… nella riva sinistra del Po, spiazzone di sabbie asciutte") (Iamonico, 2015a). In the 1980s further findings upstream and downstream the Po River were detected (Soldano, 1982; Iamonico 2015a). In 1988 and 1989, Zanotti (1989) recorded the species along the river Oglio in the province of Brescia (Lombardy). In the Veneto region, the species has been first recorded in the early 2000s (Masin & Scortegagna, 2012). *A. tuberculatus* is presently considered invasive in four regions Lombardy, Emilia-Romagna, Veneto and Marche (Banfi & Galasso, 2010; Iamonico, 2015a; Acta Plantarum, 2019). locally established/transient: Piemonte, Friuli Venezia Giulia, Tuscany (La Rosa & Peruzzi, 2013; Lazzeri *et al.*, 2013; Iamonico, 2015a; Verloove & Ardenghi, 2015). In Trentino-Alto Adige the species has been found in 2012 and 2013 (Bertolli & Prosser, 2014). In Tuscany, the species was detected in 2004 (location: swamp "Fucecchio"; La Rosa & Peruzzi 2013) and in Pisa 2013 (banks of the Arno river; Lazzeri *et al.*, 2013). For example, in Piemonte, Verloove & Ardenghi (2015) detected *A. tuberuclatus* together with *Amaranthus palmeri* along the river Scrivia (Tortona).

Germany: *Amaranthus tuberculatus* has been found occasionally in low numbers, primarily in port areas along the river Rhine (Neuss, Mannheim; Schmitz, 2002; Amarell, 2010). In the database " Florenliste von Deutschland, Gefäßpflanzen" the species is considered to be transient in Baden-Württemberg and North Rhine-Westphalia (Buttler *et al.*, 2018). Junghans (2016) who judges the species as *Amaranthus rudis*, noticed that the population in the Mannheim area seems to have established, even though new introductions from a nearby animal feed factory cannot be excluded.

Finland: Casual occurrences of *A. tuberculatus* (as *A. rudis*) have been documented near the town Turku (FinBIF, 2019).

Israel: *Amaranthus tuberculatus* (as *A. rudis*) has been found in several districts but it is considered rare (Danin & Fragman-Sapir, 2019). *A.tuberculatus* can be found near stream banks and canals in some specific spots: North: Hula valley, Jezreel Valley (Genigar, Mishmar haemek), Carmel cost (Shfeia) and in the Center: Yad Binyamin (Cafri, pers. comm., 2019). Early occurrences are from 1970 (upper Jordan valley) and 1982 (Tirat Zvi), and *A. tuberculatus* was considered to be established "… on the northern [shore] of Lake Kinnerei" (Greuter & Reus, 1986).

Jordan: *Amaranthus tuberculatus* occurs in the Jordan valley (Taifour *et al.*, 2016).

Romania: *Amaranthus tuberculatus* (as *A. tamariscinus*) has been detected by Costea (1996) between 1993 to 1995 at Constanţa harbour, which is an important entrance point of alien species in Romania. Later, Memedemin *et al.* (2016) described the species (as *A. rudis*) again at the Constanţa harbour within the observation period that lasted from 2004 to 2014. Anastasiu *et al.* (2011) have also found it along the Black Sea coastal area without any concrete information about the place of discovery.

Russian Federation: *Amaranthus tuberculatus* (and *A. rudis*) is mentioned in Czerepanov (1995) without any further details. No recent information has been found.

Spain: Anthos (2019) does not list *A. tuberculatus*, however Sánchez Gullón & Verloove (2013) described the species as transient in Huelva in port areas near grain silos and unloading quays for cereals.

Switzerland: Currently, the species is considered not to be part of the Swiss Flora, however there is one herbarium record from 1847 for Geneva available (Buholzer, pers. comm., 2019).

UK: It has been rarely found in Britain, as such in Bristol in 1958 and 1959 within the port area (Brenan, 1961). However, the species is not mentioned in the Online Atlas of the British and Irish flora (BRC, 2019).

Ukraine: According to Mosyakin & Fedoronchuk (1999), *A. tuberculatus* is a rare and a transient species. It has been found along railway tracks in Kiev and SE Ukraine. Yavorska (2009) considered *A. tuberculatus* to be an ephemerophyte.

It should be noted that there are uncertainties about the exact distribution of *A. tuberculatus* in the EPPO region due to the transient nature of the species in the region.

7. Habitats at risk and their distribution in the PRA area (habitat classification based on EUNIS habitat types)

Habitat (main)	**Classification**	**Status of habitat**	**Is the pest present in the habitat in the PRA area (Yes/No)**	**Comments (e.g. *major/minor habitats* in the PRA area)**	***Reference***
C: Inland surface waters	Temporary running waters (C2.5), Littoral zone of inland surface waterbodies (C3)	Protected in part	Yes	Major	Verloove, 2019 Iamonico, 2015a
E: Grasslands and lands dominated by forbs, mosses or lichens;*	Ruderal environments (E5.1): Hard-surfaced areas of ports (J4.5), Rail networks (J4.3)	None	Yes	Major	Verloove, 2019 Junghans, 2016
I: Regularly or recently cultivated agricultural, horticultural and domestic habitats,	Cultivated fields, bare tilled, fallow or recently abandoned arable land (I1.5)	None	Yes	Major	Banfi & Galasso, 2010

* 'ruderal or pioneer communities invading these artifical habitats' are included in E5.1 Anthropogenic Herbaceous Formations (EUNIS Habitat).

Habitat in its native range is described in section 2.5.

Suitable habitats occur for the establishment of *A. tuberculatus* in the PRA area, It occupies different environments: floodplains and banks of major rivers such as the Po, Rhine, Maas and Waal (Verloove, 2019), ruderal habitats (e.g. railway tracks, port areas; Sánchez Gullón & Verloove, 2013; Junghans, 2016), and to a lesser extent crop fields (see section 2.6) (Banfi & Galasso, 2010; Masin & Scortegagna, 2012; Pellizzari *et al.*, 2015; Iamonico, 2015a).

Some data is available on *A. tuberculatus* showing that it is able to invade natural riverside vegetation in Italy (Iamonico, 2015a).

8. Pathways for entry

Seeds and grain (as commodities) should be understood in this PRA as defined in ISPM 5 (2019)

- Seeds: seeds (in the botanical sense) for planting [FAO, 1990; revised ICPM, 2001; CPM, 2016]
- Grain: Seeds (in the botanical sense) for processing or consumption, but not for planting [FAO, 1990; revised ICPM, 2001; CPM, 2016].

A. tuberculatus has presumably been introduced in Europe as a grain contaminant. Records were from ruderal sites in port areas and along (nearby) riverbanks indicating its introduction via imported goods (grain, animal feed mixture). In addition, contamination of seeds for planting is a further pathway.

The following pathways for entry of *A. tuberculatus* are discussed in this PRA. Pathways in bold are studied in section 8.1; other pathways were considered as a very low likelihood of entry and are detailed in section 8.2.

- **Grain for animal feed mixtures, human consumption and processing purposes**
- **Seed**
- **Seed mixtures and native seeds**

- **Used agricultural machinery and equipment**
- Manure
- Natural spread
- Soil and other growing media (on its own or associated with plants for planting other than seeds)
- Intentional importation of *A. tuberculatus*
- Travellers and their equipment
- Hay

8.1 Pathways studied

All the pathways evaluated are from areas where the pest has been reported to be present, into the EPPO region. Examples of prohibition or inspection are given only for some EPPO countries (in this express PRA the regulations of all EPPO countries was not fully analysed). Similarly, the current phytosanitary requirements of EPPO countries in place on the different pathways are not detailed in this PRA (although some were taken into account when looking at management options). EPPO countries would have to check whether their current requirements are appropriate to help preventing the introduction of the pest

Pathway	**Grain (for animal feed mixtures, human consumption and processing purposes)**
Coverage (short description why it is considered a pathway)	Seeds of *A. tuberculatus* maybe a contaminant in unprocessed grains imported for animal feed mixture and human consumption. Grains for processing purposes are included from this pathway because it is considered that even though the industrial process could be partially or totally destructive, storage and transportation conditions may allow spread of *A. tuberculatus*. This pathway covers all summer grains industrially harvested in the area of origin in which *A. tuberculatus* was reported. This is limited to ***Glycine max*, *Phaseolus vulgaris* (dried grains), *Sorghum bicolor* and *Zea mays*.** Additional summer industrially harvested crops may need to be considered in the future if there is evidence that *A. tuberculatus* is associated with these crops. Grains packaged and prepared for final human consumption are not considered a pathway as contamination is considered very low and transfer to a suitable pathway is unlikely.
Pathway prohibited in the PRA area?	No. However, some EPPO countries impose import requirements on the purity of the grain for animal feed. However, some EPPO countries impose import requirements on the purity of the grain for animal feed. *Ambrosia* spp. have been added to the list of harmful botanical impurities that are included in Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Feed material and compound feed containing unground grains and seeds should contain a maximum of 50 mg of seeds of *Ambrosia* spp. per kg (relative to a feed with a moisture content of 12 %). Exceptions apply to millet (grains of *Panicum miliaceum*) and sorghum (grains of *Sorghum bicolor*) that are not directly fed to animals and which may contain a maximum of 200 mg of seeds of *Ambrosia* spp. per kg (relative to a feed with a moisture content of 12 %).
Pathway subject to a plant health inspection at import	No. The EWG was not aware of plant health regulations imposing inspection at import in the EPPO region on these commodities.
Pest already intercepted?	Yes *A. tuberculatus* has been intercepted in bird feed in the USA (Oseland *et al.*, 2020). In Israel and Romania, it is assumed that the species was introduced by fish food from North America (Greuter & Raus 1986) and with soybean waste and cereals (Costea, 1996), respectively. In Belgium, *A. tuberculatus* is usually found under grain conveyors, near grain mills, on unloading quays or along road verges. The weed is also observed growing from soybean waste (http://alienplantsbelgium.be/content/amaranthus-tuberculatus). Moreover, *Amaranthus* spp. may be a grain contaminant. In Canada, different *Amaranthus* spp. have been intercepted in grain of maize, soybean, cereals, pulses, canola, sunflower and millet from the USA between 2007 and 2015 (Wilson *et al.*, 2016). Shimono & Komuna (2008) showed a contamination of spring wheat destined for milling for human food trade imported from Canada to Japan with *A. retroflexus*. *A. retroflexus* and *A. hybridus* have also been intercepted in the Netherlands for fodder and birdseed from North America (van Denderen *et al.*, 2010). *A. palmeri* has also been intercepted in grain commodities (EPPO, 2020b).

Pathway	Grain (for animal feed mixtures, human consumption and processing purposes)
Most likely stages associated with the pathway	In North America, *A. tuberculatus* is a weed of many crops in particular maize and soybean (Costea *et al.*, 2005; Schryver *et al.*, 20017; Sarangi & Jahla, 2018). Seeds of *A. tuberculatus* may become associated with seeds of summer crops at harvest where the species occurs.
Important factors for association with the pathway	Association depends on the exact origin of the imported product and the degree of infestation of this region with *A. tuberculatus* Mixture of grains from different origins present a higher risk of contamination because of lack of traceability. Bird seeds are often composed of different grain species, among which maize and sorghum are crops that *A. tuberculatus* is known to infest. The most common grains found are black or striped sunflower seeds, decorticated sunflower, wheat, barley, (hulled) oats, millet, sorghum, Niger seed, (cracked) maize, safflower, groundnut or groundnut pieces, pine nuts, canary seed and quinoa. Some companies include in their product range special mixes that are intended to attract particular groups or species of birds (e.g. Niger seed to attract finches, peanuts and other large seeded grains for woodpeckers and nuthatches) (FAO, 2005). Association also depends on the use of the commodity and the cleaning performed before exportation: - The grains imported for human consumption are likely to be less contaminated than grains for animal consumption as grains for human consumption are cleaned before export to a very high standard to ensure quality and consistency for the end product. - The processing of grain for animal feed has less restrictive standards than for human consumption, and therefore such grains may be cleaned and processed to a lesser degree. Therefore, although the probability of entry into the EPPO region would be the same for both human consumption and animal feed, differences methods in processing should be taken into account. The timing of harvest can influence if *A. tuberculatus* contaminates the commodity. Schwartz *et al.* (2016) showed that more than 95% of the seeds of *A. tuberculatus* were still attached to the plant at soybean maturity. Seed that have been shed from plants at harvest are less likely to enter the grain pathway. The likelihood that *A. tuberculatus* seeds are associated with the pathway at origin greatly depends on the effectiveness of the management measures implemented during cultivation. Seeds of *A. tuberculatus* are small and can be easily missed with just visual examination of the commodity alone. Some national regulations impose that bird seeds are devitalised before being commercialized. This process renders seed inviable and can be achieved by techniques such as heat treatment, irradiation, physically crushing or steaming of the seeds (Blythman & Sansom, 2019).

Pathway	Grain (for animal feed mixtures, human consumption and processing purposes)
	Grain lots may be sorted before processing to remove external matters such as weed seeds. If the sorting is performed in the exporting country, especially when the size and/or colour of the seed is very different from those of *A. tuberculatus* seeds, this will reduce the association with the pathway.
Survival during transport and storage	The seeds of *A. tuberculatus* can remain viable for many years (Burnside *et al.*, 1996; Buhler & Hartzler, 2001) enabling their survival along the pathway. However, the processing procedure may act to crush and destroy the seeds. In some cases, the seeds may stay whole and survive.
Trade	There is a trade of grain (animal feed and human consumption) from countries where the pest occurs into the EPPO region. The figures in appendix 6 (from FAOStat, imports reported by EPPO countries) give an indication of the existence of a trade for soybean and maize.
Will the volume of movement along the pathway support entry?	It is likely that the movement of current volumes of the commodity, will support entry. Appendix 6 shows volumes of grain (soybean and maize) entering the EPPO region from USA. Potentially, these figures may contain volumes for various uses (including potential industrial use), but the main volume would be for animal feed or human consumption. The figures for soybean and maize grain imports show a high volume and reasonably consistent volume of import from the USA into the EPPO region.
Will the frequency of movement along the pathway support entry?	The EWG consider that the frequency of movement along the pathway is likely to support entry. Although there are no figures to highlight the frequency of movement of *A. tuberculatus* seeds as a contaminant of grain it is likely that movement with volumes of the commodities will support entry. Grain is frequently imported into the EPPO region from the USA (see Appendix 6). However, although the frequency varies year on year, the frequency of grain imports is regular, with equivalent volumes each year.
Transfer to a suitable habitat	Grain may be directly placed in suitable habitats to feed livestock or in gardens to feed bird (Blythman & Sansom, 2019) or in meadows or along agricultural fields to feed game animals for hunting. *Amaranthus tuberculatus* has been reported established near the feeding areas on the banks of fish ponds (di Castri *et al*, 1990). Grain can be transferred to a suitable habitat via the ingestion of seed by animals and depositing of feces that contain viable seeds. *Amaranthus* seeds have been shown to remain viable following the ingestion process in animals and birds. Storage and transport conditions of grains for livestock or industrial processing may also allow further spread of *A. tuberculatus* (e.g. along roads). During loading, transportation and unloading of grains, any *A. tuberculatus* seeds falling to the ground could lead to an established population, as a shown by species' records in such sites (Junghans, 2016; Verloove, 2019). However, in the areas of introduction such as ports, airports or freight stations where cargos of seed for sowing or grain for industry or livestock pass through, *A. tuberculatus* seeds would have

Pathway	Grain (for animal feed mixtures, human consumption and processing purposes)
	more difficulty becoming established because of the presence of concrete instead of soil and because of the possible management of weeds in these areas. Grain lots may be sorted before processing. If the sorting is performed after exportation, and the waste from the sorting is put in fields, they may become infested.
Likelihood of entry and uncertainty	The EWG recommended to divide the grain pathways because of the different risk of *A. tuberculatus* reaching the natural environment with these commodities: Grains for animal feed - Grains for livestock (*Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*): High likelihood of entry (high volumes, reports of association and entry of other *Amaranthus* with this pathway, less quality grains than for human consumption, used in a suitable habitat; however, sorting applied and effective due to the difference of size of *A. tuberculatus* seeds with the grains for livestock), with a moderate uncertainty (uncertainty about the production process). - Bird feed: Very high likelihood of entry (evidences that bird seeds are contaminated, mixes of grains often of lower quality, used in a suitable habitat), with a high uncertainty (uncertainty about the volume of trade, whether seeds are mixed before or after exportation; no evidence of entry with this pathway) Grains for human consumption and processing purposes (*Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*): Low likelihood of entry (higher quality standard, not for use directly in a suitable habitat: for consumption or processing, transient reports in port areas), with a moderate uncertainty (different quality standards of grains for further processing in the EPPO region).

Pathway	Seed
Coverage (short description why it is considered a pathway)	This pathway covers not only certified but also uncertified seeds. This is limited to seed lots of ***Beta vulgaris, Glycine max, Gossypium hirsutum, Medicago sativa, Phaseolus vulgaris, Sorghum bicolor*** **and *Zea mays***. *Amaranthus tuberculatus* infests many crops, in particular maize and soybean (Sarangi & Jahla, 2018) and these crops are harvested at a period when seeds of *A. tuberculatus* are present. Seed lots can therefore be infested by seeds of *A. tuberculatus*. Additional summer industrially harvested seeds may need to be considered in the future if there is evidence that *A. tuberculatus* is associated with these crops. The seed mixes of other species are treated separately due to the lack of information on species composition and traded volume are lacking to fully assess and rate this pathway.
Pathway prohibited in the PRA area?	No, this pathway is not prohibited in the PRA area. However, some EPPO countries impose import requirements which should contribute to the reduction of the number of *A. tuberculatus* seeds in the imported seed consignments (for example, at the EU level, in marketing Directives for seeds https://ec.europa.eu/food/plant/plant_propagation_material/legislation/eu_marketing_requirements_en). In particular, cereal seeds (including *Sorghum bicolor* and *Zea mays* seeds, except popcorn and sweet corn), oil and fibre seeds (including *Glycine max* and *Gossypium* spp. seeds) fodder plant seeds (including *Medicago sativa*) and beet seeds (i.e. *Beta vulgaris*) can only be imported from third countries into the EU if an equivalence with certification production conditions in the EU has been granted. The marketing of certified seeds includes purity requirements. - For *Zea mays* seeds, an examination of the seed samples is performed to guarantee that zero seeds of other plant species in a sample of 250g of basic seeds of inbreed lines; or in 1kg for other basic seeds and certified seeds, are present. (Council directive 66/402/EEC of 14 June 1966 on the marketing of cereal seeds). - For *Sorghum bicolor*, an examination of the seed samples is performed to guarantee that less than 4 seeds of other plant species in a sample of 900g of basic seeds; or 10 seeds in a sample of 500g for certified seeds of 1st and 2nd category are present. (Council directive 66/402/EEC of 14 June 1966 on the marketing of cereal seeds). - For *Glycine max* seeds, the maximum tolerance is 5 seeds of other plants in 1kg of seeds (Council directive 2002/57/EC on the marketing of seed of oil and fibre plants). - For *Gossypium* spp. seeds, the maximum tolerance is 15 seeds of other plants in 1kg of seeds (Council directive 2002/57/EC on the marketing of seed of oil and fibre plants). - For *Beta vulgaris*, the percentage by weight of other seeds shall not exceed 0,3 in a sample of 500 g of seeds (Council directive 2002/54/EC on the marketing of beet seed). - For *Medicago sativa*, the maximum tolerance is 20 seeds of another plant species in 300g of seeds (Council directive 66/401/EEC on the marketing of fodder plant seed)

Pathway	**Seed**
Pathway subject to a plant health inspection at import?	Partly. In some EPPO countries. For example, seeds of *Sorghum*, *Glycine max* imported from all third countries into the EU and seeds of *Beta vulgaris*, *Gossypium*, *Medicago sativa* and *Zea mays* from third countries other than Switzerland are subject to a phytosanitary certificate (Regulation 2019/2072), and to a plant health inspection upon arrival of the consignment at the border control post. Those official controls shall include physical checks, at a frequency depending on the risk (article 49 of 2017/625 Official control regulation).
Pest already intercepted?	No, to date, *A. tuberculatus* has not been intercepted along this pathway. However, both Canadian Food Inspection Agency (2018) and PPQ (2019) highlight the movement of other *Amaranthus* species (*A. palmeri)* seed as a contaminant of seed. *Amaranthus palmeri* has also been identified from certified soybean in seed lots and seed bags in Louisiana (pers comm. J. Ferrell, 2020). Uncertified commercial seeds from Australia, USA and Europe (e.g. novel forage seeds) have been demonstrated to harbour seed contaminants, including several *Amaranthaceae* species (Cossu *et al*, 2019)
Most likely stages associated with the pathway	Seeds of *A. tuberculatus* may become associated with seeds of crops (e.g. maize, soybean) at harvest.
Important factors for association with the pathway	The probability that seeds of *A. tuberculatus* are associated with the pathway at origin depends mainly on the crop species concerned (summer crops are more likely to be contaminated), on the exact origin of the imported product and the degree of infestation of this region by *A. tuberculatus*. The timing of harvest can influence if *A. tuberculatus* contaminates the commodity. Schwartz *et al.* (2016) showed that more than 95% of the seeds of *A. tuberculatus* were still attached to the plant at soybean maturity The likelihood that *A. tuberculatus* seeds are associated with the pathway at the point of origin greatly depends on the effectiveness of the management measures implemented during cultivation, the degree of herbicide resistance of local populations, and the cleaning procedures that are implemented at the origin before export. Seeds may be sorted after harvest and submitted to quality requirements in particular when they are certified, which will reduce the probability of association (EU marketing directives, OECD Standards). Seeds of *A. tuberculatus* are small (0.7-1 mm) in relation to the commercial seeds imported for planting in agriculture (e.g. maize and soybean). This size difference would facilitate the successful sorting process when performed. However, when performed, physical checks may not allow to detect the presence of *A. tuberculatus* seeds in the consignment.
Survival during transport and storage	The seeds of *A. tuberculatus* can remain viable for many years (Burnside *et al.*, 1996; Buhler & Hartzler, 2001) enabling their survival along the pathway.
Trade	There is a trade of seed (for planting) from countries where the pest occurs into the EPPO region. The figures in appendix 7 (from FAOStat, imports reported by EPPO countries) give an indication of the existence of a trade for seed of maize, sorghum and soybean.

Pathway	Seed
Will the volume of movement along the pathway support entry?	Yes, Appendix 7 provides figures on the quantities of maize, sorghum and soybean imported into the EPPO region from the USA from 2015-2018. Although there is variation year on year, there are significant volumes of the aforementioned seed entering the EPPO region. The EWG consider, it is likely that the volume of *A. tuberculatus* as a contaminant along this pathway will be proportionate to imports into the PRA area as seeds are expected to come from areas that are heavily infested by *A tuberculatus*.
Will the frequency of movement along the pathway support entry?	As mentioned, although the frequency of movement of maize, sorghum and soybean imported into the EPPO region from the USA, varies year on year, the frequency of seed imports is regular, with equivalent volumes each year (an increase for maize, a decrease for soybeans). The frequency of movements along the pathway has no impact on the viability of the seeds introduced or on their quantity. Only the volumes imported can have an impact on the likelihood of introduction.
Transfer to a suitable habitat	Seed for sowing contaminated by *A. tuberculatus* is directly sown in agricultural fields, which is an optimal habitat for this species.
Likelihood of entry and uncertainty	Seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*: Moderate likelihood of entry (used in a very suitable habitat, reports of association with the pathway for other *Amaranthus* species, reports of presence in crop fields in the EPPO region; but quality certification standards) with Moderate uncertainty (uncertainty about the source of entry in agricultural crops in Italy, uncertainty about efficiency of the sorting/cleaning process, uncertainty about the use of certified vs. non-certified seeds by EPPO countries)

- **Seeds mixtures and native seeds**

No reports of presence of *A. tuberculatus* in seed mixtures and native seeds from North America has been found; however, this has been reported for other *Amaranthus* species (including *A. palmeri*) and the EWG considered that this is also probably the case for *A. tuberculatus*. Indeed, USDA (2013) details that *A. palmeri* was identified as a contaminant in conservation plantings in Illinois, Indiana, Iowa, Minnesota and Ohio. It was a contaminant in Conservation Reserve Program (CRP) seed mixes. Some native seed mixes planted to foster habitats for honeybees and other pollinators have been found to be contaminated with *A. palmeri* (WSU, 2020). *A. palmeri* was also found in crop pollinator commercial seed mixtures in the USA (Oseland *et al.*, 2017). Additionally, seed mixtures for conservation, pollination and seed mixtures for forage plants game animals (for example see: https://www.plantbiologic.com/products/last-bite-food-plot-seed) will be placed directly in habitats that are suitable for *Amaranthus* species. However, data on the seed species composition present in the seed mixtures that were intercepted is lacking. In some EPPO countries (e.g. the EU), all imported seeds should be accompanied with a phytosanitary certificate mentioning the seed species included in the mixture (Regulation EU 2016/2031). However, it may not be the case for every EPPO countries. Seed mixtures may have very variable composition. Individual species are typically produced in agricultural fields and mixed afterwards (Hartzler, pers. comm., 2020). Information on traded volume is lacking; however, the EWG considered that such mixtures are imported in lower quantities than seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*.

Likelihood of entry and uncertainty: Moderate (lower volume than the seed pathway) with a high uncertainty (different uses, origin of the mixes used in the EPPO region, composition of the mixes).

- **Used agricultural machinery and equipment.** Seed of *A. tuberculatus* may become a contaminant of machinery and equipment. This pathway may play a role for local and/or cross border spread. Schryver *et al.* (2017) stated that *A. tuberculatus* was most likely introduced from Illinois/USA to Ontario/Canada in the 1990s and early 2000s via a contaminated demonstration combine. Data is lacking to fully assess and rate this pathway. However, there is probably very little movement of used machinery from the countries where the pest occurs into the EPPO region and if there is, it is probable that such equipment would undergo phytosanitary procedures such as decontamination (e.g. in the EU, machinery and vehicles imported from third countries other than Switzerland and which have been operated for agricultural or forestry purposes should be cleaned and free from soil and plant debris (Regulation (EU) 2019/2072)). The EWG considered that due to the small size of *A. tuberculatus* seeds, cleaning procedures applied may not be fully effective, in particular for harvest combines. Agricultural machinery will likely be used in suitable habitats. A few seeds can start a new population. This pathway is covered by an International Standard for Phytosanitary Measures (ISPM 41) (IPPC, 2017a).

Likelihood of entry and uncertainty: High (size of the seeds, difficulty to clean some machinery and equipment, may be higher for some countries without a market for agricultural machinery or involved in cooperation programs) with a high uncertainty (Volume and frequency of movement).

Overall rating of the likelihood of entry combining the assessments from the individual pathways considered:

Rating of the overall likelihood of entry		*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☐	*Very high* X
Rating of uncertainty				*Low* ☐	*Moderate* X	*High* ☐

8.2 Pathways with a very low likelihood of entry:

The uncertainty was assessed to be low for all pathways below.

- **Manure.** Costea *et al*., (2005) mentioned that *A. tuberculatus* can be associated with manure. However, the movement of manure from the USA to the EPPO region is likely to be extremely low.

- **Natural spread**. Apart from EPPO countries detailed in section 7, *A. tuberculatus* is present only in North America. The species would not spread naturally from North America into the EPPO region.

- **Soil and other growing media (on its own or associated with plants for planting other than seeds).** From countries where the pest occurs (see ISPM 40): import of growing media is prohibited in most EPPO countries (e.g. importation of soil and growing medium as such is prohibited in the EU, and is regulated when associated with plants (Regulation (EU) 2019/2072)) and therefore there is a very low likelihood of entry as a contaminant on this pathway.

- **Intentional importation of *A. tuberculatus*.** Trade of *A. tuberculatus* is unlikely as it is not usually used or traded as an ornamental species or for other uses. *A. tuberculatus* could be imported for research purposes.

- **Travellers and their equipment.** *A. tuberculatus* seeds may be a contaminant of travellers and their equipment (e.g. shoes, clothes and leisure equipment (tents, bags, etc.)). Data is lacking to fully assess this pathway but the EWG considered that there was a very low likelihood of entry.

- **Hay.** Both USDA (2019b) and Canadian Food Inspection Agency (2018) detail the potential of movement of another *Amaranthus* species, *A. palmeri*, as a contaminant of hay material for the USA and Canada, respectively. FAO (2020) provides limited data on the export of hay from the USA to the EPPO region, where Austria, Finland, Norway, Sweden and Tunisia are reported to have received imports between 2012 -2017 under the item code 859 Hay (unspecified). *A. tuberculatus* is not growing well in pastures and hay fields (high uncertainty (absence of reports)).

9. Likelihood of establishment outdoors in the PRA area

Habitats detailed in section 7 are widespread within the EPPO region and thus further establishment is likely in areas where climatic conditions are conducive for establishment.

9.1 Natural habitats

Amaranthus tuberculatus is already established in Italy and Israel in the natural environment, in particular in disturbed habitats.

Populations of *A. tuberculatus* can already be found along riverbanks in several EPPO countries (Iamonico, 2015a; Verloove, 2019). In stable, intact natural habitats interspecies competition and the late emergence pattern may limit establishment.

Most natural habitats of high conservation value are unsuitable for *A. tuberculatus*, and thus negative effects of this plant on biodiversity are considered to be of low importance (Iamonico, 2015a)..

9.2 Managed habitats

It is likely that *A. tuberculatus* can establish in the managed environment. It is capable of rapidly invading disturbed areas because of copious seed production and the formation of a persistent seed bank.

In ruderal and agricultural environments, it is unlikely that competition with cultivated plants would prevent the establishment of the species. *A. tuberculatus* is capable of invading many summer crops in particular late sowing crops like maize and soybean. However, high frequency of maize and soybean in the crop rotation system in many EPPO countries is a factor that may strongly endorse the establishment of *A. tuberculatus* once the field has become contaminated. Reduced tillage is likely to promote *A. tuberculatus* because seeds remain near the soil surface, which promotes germination and emergence (Costea *et al.*, 2005; Schryver *et al.*, 2017).

In crops, common weed control methods may not be sufficient to limit the development of the species due to discontinuous emergence pattern and rapid growth. Further complications may arise from the reduction in the number of herbicide compounds (in particular compounds with residual soil activity), the herbicide resistance against multiple mode of actions in this species (Sarangi *et al.*, 2019) and the decrease in the number of herbicides treatments associated with the reduction in the use of plant protection products. All of the aforementioned factors can potentially foster the establishment of *A. tuberculatus*.

In areas where the climatic conditions are suitable for the establishment of the species, establishment can occur along roadsides, railway networks, ports area (e.g. in Spain) etc. These habitats may act to promote the spread of the species into other managed habitats in close proximity (e.g. agricultural fields). Public and private gardens may also faciliate the establihsment.

9.3 Other factors affecting establishment

Natural enemies

Within the EPPO region, there are no host specific natural enemies of *A. tuberculatus*. Generalist natural enemies will potentially attack the plant, but these are unlikely to inflect enough damage at the population level to influence establishment.

Abiotic factors

- **Climate conditions**

The major factors that could limit the establishment of *A. tuberculatus* in the EPPO region are assumed to be low summer temperatures and low moisture. Extreme low values at locations where the species is known to occur (1% quantile) are 18°C for summer temperatures and precipitation is 30% of potential evapotranspiration (PET). (Appendix 2).

A. tuberculatus is a frost sensitive species (Costea *et al*., 2005) and this may prevent its establishment in higher latitudinal areas of the EPPO region, especially where the growing season is short, and frost may kill the plant before seed set.

The potential distribution in the EPPO region is limited by the low summer temperatures in the North and drought stress in the South.

The species distribution modelling shows that *A. tuberculatus* could establish in all countries bordering the Mediterranean sea, especially in the agricultural production areas of Iberia (Spain and Portugal), South of France, Italy, Adriatic coast (incl. Croatia), as well as the Pannonian Basin and countries bordering the Black Sea.

With moderate and extreme climate change scenarios (RCP 4.5 and 8.5), the projected distribution may expand within the EPPO region as far as the southern Baltic Sea coast (Appendix 2).

- **Soil conditions**

Amaranthus tuberculatus can tolerate a wide range of soil types and textures preferring nutrient rich soils.

Rating of the likelihood of establishment outdoors in the PRA area	Very low ☐	Low ☐	Moderate ☐	High X	Very high X
Rating of uncertainty			*Low* X	*Moderate* ☐	*High* ☐

The EWG considered that this rating applied to the area of potential establishment. The rating would be lower for other areas of the EPPO region.

10. Likelihood of establishment in protected conditions in the PRA area

No evidence was found of the presence of *A. tuberculatus* under protected conditions in North America.

The management of temperatures under protection (e.g. polytunnels, glasshouses) maintains average temperatures between 20 and 35 °C which would be more favourable for the development of the species. Protected conditions, such as in nurseries, polytunnels, tropical greenhouses may offer appropriate conditions for the development of the pest.

However, these crops are often produced in highly managed production systems (with possible rotation relocation e.g. for polytunnels) that would limit the likelihood of establishment due to short intervals between consecutive management practices.

Rating of the likelihood of establishment in projected conditions in the PRA area	Very low ☐	Low ☐	Moderate X	High ☐	Very high ☐
Rating of uncertainty			Low ☐	Moderate ☐	High X

Uncertainty: level of management applied, variability of production systems, lack of observation of A. tuberculatus *in such conditions.*

11. Spread in the PRA area

Natural spread

There is no specific information regarding natural seed dispersal of *A. tuberculatus* (Costea *et al.*, 2005). The species can produce a large amount of seeds which are light in weight, though they have no special adaptions for wind-dispersal. Seeds are assumed to fall near the mother plant. However, natural local dispersal is most likely accomplished by water, as with other *Amaranthus* spp. as both seeds and fruits can float easily (Costea *et al.* 2004). Seeds may be dispersed by rain drops and streamlets produced on the soil by rain.

In addition, *Amaranthus* species can be spread by animal species e.g. bird species (Costea *et al.*, 2005; Farmer *et al*. 2017; Ward *et al*., 2013). *Amaranthus* seeds can maintain viability when moving through the digestive tract of birds (de Vlaming & Proctor, 1968; PPQ, 2019). Additionally, mice, rabbits, sheep and cattle can ingest and spread *Amaranthus* seeds (PPQ, 2019).

In the EPPO region, spread of *A. tuberculatus* has been observed along river systems in Northern Italy (e.g. Po river, Arno river and Oglio river) (Section 6) (Iamonico, 2015a).

Human assisted spread

Seeds of *A. tuberculatus* can be spread by agricultural machinery and equipment by contaminated soil attached to disc, harrow or plough (Costea *et al.*, 2005). As seeds are still attached to the plant at the time of harvest, *A. tuberculatus* can be dispersed by combine harvesters, which may then transfer the seeds from field to field.

Schwartz *et al.* (2016) showed that more than 95% of the seeds of *A. tuberculatus* were still attached to the plant at soybean maturity (Nebraska, Missouri, Wisconsin, Illinois/ USA). Schryver *et al.* (2017) stated that *A. tuberculatus* was most likely introduced from Illinois/USA to Ontario/Canada in the 1990s and early 2000s via a contaminated demonstration combine. Crop residues, compost, straw, hay, and manure may contain *Amaranthus* spp. seeds and contribute to its spread from field-to-field and within-field (Eberlein *et al.*, 1992; Costea *et al.*, 2005). Seeds may be spread through surface irrigation within a field and from field to field.

This has been shown for its congener *Amaranthus palmeri* (Norsworthy *et al.*, 2014.). In this research, it took only 20,000 seed initially introduced into one m^2 to effectively colonize 0.53- to 0.77-ha fields in less than 2 years. It is believed by the authors that rainwater and harvesting equipment dispersed the seeds from the original area of introduction.

There is limited evidence of *A. tuberculatus* spread by human-assisted means within the EPPO region. However, Bertolli & Prosser (2014) suggest the species has spread via forage material coming from the Po valley often used in Trentino stables. Although *A. tuberculatus* is not widespread and is not expected to establish in agricultural areas of the EPPO region initially, spread is expected in ruderal and agricultural habitats as observed in North America. With a future increase of populations in agricultural areas in the EPPO region, spread will be highly facilitated by movement of machineries from farm to farm. Spread of *A. tuberculatus* in the EPPO region will also be facilitated by contamination and either subsequent spillage from transporting containers of crops intended for seed or grain for processing or feed for livestock or wild animals. A large range of expansion is expected in the EPPO region, when *A. tuberculatus* will be found in higher quantities in agricultural fields.

Rating of the magnitude of spread in the PRA area	Very low ☐	Low ☐	Moderate ☐	High ☐	Very high X
Rating of uncertainty			Low ☐	Moderate X	High ☐

The EWG noted that the uncertainty will decrease in the future if A. tuberculatus *is found to be present in higher quantities in agricultural fields*

Uncertainty: presence in agricultural fields

12. Impact in the current area of distribution

12.1 Impacts on biodiversity

There are no scientific studies on negative biodiversity impacts in North America.

In the EPPO region, observational data shows that *A. tuberculatus* invades riparian habitat. In Italy, it occurs mainly on floodplains and banks of the rivers Po, Arno, Piave, Metauro and of numerous smaller watercourses forming dense and extensive populations (Iamonico, 2015a). A negative impact of *A. tuberculatus* has been reported from Italy along the Po River (Iamonico, 2015a), however scientific studies quantifying the impact are not available.

12.2 Impact on ecosystem services

There is no evidence that *A. tuberculatus* has negative impacts on supporting and cultural ecosystem services.

Provisioning and regulating ecosystem services are dealt in section 12.3.

12.3 Socio-economic impact

Amaranthus tuberculatus already occurs very locally in crop fields in the EPPO region (Italy: Pellizzari, *et al*. 2015; Iamonico, 2015a), e.g. on muddy soils in Veneto (Masin & Scortegagna, 2012). Specific studies on yield loss or additional operating costs are not available for the EPPO region.

Detailed information on socio-economic impacts is available from North America. The economic consequences associated with the presence of *A. tuberculatus* are considered major from an agricultural and public health point of view.

Agriculture

It is a competitive annual weeds in maize, soybean and cotton in the United States Corn Belt and Canada (Sarangi & Jahla, 2016; Schryver *et al*., 2017; Sarangi *et al*., 2019; Werner *et al*., 2019), though competitiveness varies with density and time of emergence relative to the crop (Bensch *et al*., 2003).

Studies focused mainly on competition between A. *tuberculatus* and a single crop. Experimental data have shown than unmanaged fields (e.g. of maize and soybean) would cause significant yield losses in these crops:
Steckel & Sprague (2004) reported that season-long interference of *A. tuberculatus* at 270 plants/m^2 can reduce maize yield by 74% (Illinois/USA). Jones *et al.* (1998) reported that *A. tuberculatus* emerging with soybean caused yield losses of 5 to 18% at densities of 7.9 and 31.5 plants/m^2, respectively. A study from Hager *et al.* (2002) reported that *A. tuberculatus* allowed to compete with soybean up to 10 weeks after soybean unifoliate expansion at a density up to 362 plants/m^2 reduced soybean yield by 43% (Illinois/USA).

In Canada, interference of *A. tuberculatus* resulted in soybean yield losses of up to 73% in weedy versus weed-free checks (Vyn *et al*., 2007). A study by Cordes *et al.* (2004) reported a maize yield loss of 36% occurred with *A. tuberculatus* density ranging from 369–445 plants/m^2 full-season interference (Missouri/USA).

Bensch *et al.* (2003) described the effect of the density of *A. tuberculatus* on soybean yield loss using a rectangular hyperbola model (Kansas/USA). Soybean yield loss varied depending on year and location between from 27 to 63%. Maximum soybean yield loss occurred at eight plants/m of row length and was 56 % for *A. tuberculatus* as determined by the model. Even the competitive impact of late emerging individuals can result in a 10% reduction in soybean yield (Bensch *et al*., 2003).

An important problem is also the evolution of herbicide-resistant *A. tuberculatus* biotypes (Sarangi *et al*., 2019). Resistant biotypes have been confirmed in populations of the species to seven different herbicide mechanisms of action including: ALS-inhibiting herbicides (e.g. imazethapyr), auxins (e.g. 2,4-D), EPSPS (e.g. glyphosate), HPPD inhibitors (e.g. mesotrione), protoporphyrinogen oxidase (PPO, e.g. acifluorfen), photosystem II (PSII, e.g. atrazine) and VLCFA (e.g. metolachlor) (Oliveira *et al*., 2018, HEAP, 2019; Sarangi *et al.* 2019). Many populations of *A. tuberculatus* contain more than one of these resistances and thus, severely limit the options for effective herbicide control. According to Sarangi *et al.* (2019), the dioecious nature of *A. tuberculatus* promotes the spread of herbicide-resistant traits through pollen-mediated gene flow. Furthermore, an individual *A. tuberculatus* female plant can produce over a million seeds (Hartzler *et al*., 2004). Thus, herbicide resistance may evolve and spread faster in *A. tuberculatus* than in other monoecious weedy *Amaranthus* spp. The species is classified among the worst herbicide resistant weeds (HEAP, 2019).

However, *A. tuberculatus* is less competitive than *A. palmeri*. Horak & Loughin (2000) reported on competitiveness (various growth parameters like dry weight, leaf area, height, etc.) of four *Amaranthus* species and ranked them as *A. palmeri* > *A. tuberculatus* > *A. retrofleuxs* > *A. albus.* (Bensch *et al*., 2003).

Bensch *et al* (2003) reported in a study that *A. tuberculatus* caused greater yield losses than *A. retroflexus*, but was not as competitive as *A. palmeri*.

Even with the figures documented on yield losses, the EWG considered that the majority of farmers are able to manage the weeds sufficiently in practice with intensive herbicide programs. This minimizes the impact on yields, and the primary negative consequences are requirements for more intensive management strategies (e.g. herbicide expenses).

Public health

Amaranthus spp. are prolific pollen producer and all pollen types are supposed to be allergenic (e.g. Wurtzen *et al.*, 1995). Thus, they should be considered as "hay fever plants" in areas where they are abundant (Oh, 2018). *A. tuberculatus* is regarded a "severe allergen" according to PollenLibrary.com (http://www.pollenlibrary.com/Specie/Amaranthus+tuberculatus/).

However, few specific studies on its health impact have been reported to date (e.g. Lewis & Imber, 1975). The authors showed that *A. tuberculatus* (considered as "western water hemp") is the worst "hay fever plant" around St. Louis (Missouri/USA). It has been indicated that 40.2% of 251 adults in the St. Louis area had a positive reaction to a skin-prick-test of *A. tuberculatus*.

Rating of the magnitude of impact in the current area of distribution	Very low ☐	Low ☐	Moderate ☐	High X	Very high ☐
Rating of uncertainty			*Low* X	*Moderate* ☐	*High* ☐

The above rating was based mainly on the data from North America.

13. Potential impact in the PRA area

13.1 Potential impacts on biodiversity in the PRA area

There is the potential for impacts on biodiversity in meso-hygroscopic environments (riverbanks, wet grasslands). There is no evidence that *A. tuberculatus* invades natural areas with high conservation value in the EPPO region.

A. tuberculatus can hybridize with other *Amaranthus* species (Section 2.3), thus adversely affecting the gene pools of other species. Hybridization is also a route by which herbicide resistance can be moved between different *Amaranthus* spp. (Costea *et al*., 2005). However, native European *Amaranthus* species are monoecious (Steckel, 2007) and are not expected to hybridize in field conditions with *A. tuberculatus* when present in a limited number.

13.2 Potential impact on ecosystem services in the PRA area

There is no evidence that *A. tuberculatus* has negative impacts on supporting and cultural ecosystem services and the EWG considered that any impact in the future will be limited to managed areas.

13.3 Potential socio-economic impact in the PRA area

Agriculture

The potential economic impact of *A. tuberculatus* in the EPPO region for farmers could be significant if the species spreads and establishes in further areas. The studies conducted in North America (chapter 12.3) indicate the degree to which *A. tuberculatus* impacts crop yield. Thus, effective weed control is essential in *A. tuberculatus* infested fields.

In general, A. *tuberculatus* can be managed in crops like other weeds by herbicide use, mechanical control and integrated pest management. Noteworthy, *A. tuberculatus* has a prolonged emergence pattern throughout the crop growing season and thus, evades weed control attempts. The species will most likely show the same behaviour in the EPPO region. Seedlings will likely establish after initial post-emergence herbicide applications and mechanical weed control tactics, therefore requiring additional weed management actions throughout the crops life span and this could raise control costs. The introduction of herbicide-resistant genotypes of *A. tuberculatus* appears high and such an introduction may indeed severely limit the options for effective herbicide control and would result in an increase in control costs due to the adoption of specific herbicide programs (e.g. Meyer *et al.* 2015).

Without the implementation of integrated control against this species – effective chemical weed control, rotation including winter crops and appropriate tillage – the negative effects of *A. tuberculatus* will probably increase. Effective chemical control options (e.g. post-emergence herbicides in soyabean in the EU) may be limited within the EPPO region due to the decrease of the number of herbicides available in the context of legislation, and due to the species being resistant to a number of active ingredients (see section 2.9).

Human health
If significant *A. tuberculatus* populations become established in the PRA area in either cultivated or uncultivated areas, the substantial pollen production may contribute to allergic rhinitis caused by its pollen. However, allergy impacts specific to *A. tuberculatus* have not been recorded in the EPPO region to date and such impact is not foreseen to be as important as other invasive alien plants (e.g. *Ambrosia* species)

Will impacts be largely the same as in the current area of distribution? Yes

It is considered that the absence of herbicide solutions in the EPPO region may be counterbalanced by greater diversity in weed management practices (e.g. tillage, crop rotation, mechanical weed control).
It is considered that uncertainty is Moderate in the EPPO region because of different production practices compared to the USA and because of absence of impact reported yet in the EPPO region.

14. Identification of the endangered area

The EWG considered that the endangered area includes agricultural environments situated to the North and east of the Mediterranean sea, especially in the agricultural production areas in Spain and Portugal, South of France, Italy, Adriatic coast (incl. Croatia), as well as the Pannonian Basin and countries bordering the Black Sea and in central Asia (appendix 3, Fig. 5). Appendix 3 gives the percentage of suitable areas in each country.

15. Overall assessment of risk

The likelihood of new introductions to the EPPO region occurring via grain of *Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays* is high with a moderate uncertainty. For seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*, the likelihood of new introductions is moderate with moderate uncertainty. Entry into the EPPO region via seed mixtures and native seeds is moderate with a high uncertainty.

The likelihood of further establishment outdoors is high with a low uncertainty. Establishment in protected conditions is medium with a high uncertainty. Protected conditions, such as in nurseries and polytunnels, may offer appropriate conditions for the development of the pest. The potential for spread within the EPPO region is very high with a moderate uncertainty. *A. tuberculatus* can spread both naturally and via human-assisted spread. Seeds of *A. tuberculatus* can be moved through agricultural machinery and products (e.g. grains, seeds) within the EPPO region.

The impacts of *A. tuberculatus* in North America are primarily the reduction of crop yields and increased management costs. The EWG considered the potential socio-economic impacts in the EPPO region will be high with a moderate uncertainty.

	Likelihood	**Uncertainty**
Entry	**Very High**	**Moderate**
Grains for animal feed, human consumption and processing purposes (*Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*)		
Grains for livestock	High	Moderate
Bird feed	Very high	High
Grains for human consumption and processing purposes	Low	Moderate
Seeds (*Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*)	Moderate	Moderate
Seed mixtures and native seeds	Moderate	High
Used agricultural machinery and equipment	High	High
Establishment outdoors in the PRA area	**Very high**	**Low**
Establishment in protected conditions in the PRA area	**Moderate**	**High**
Spread	**Very High**	**Moderate**
Impact in the current area of distribution	**High**	**Low**
Potential impact in the PRA area	**High**	**Moderate**

Stage 3. Pest risk management

16. Phytosanitary measures

The EWG considered that phytosanitary measures should be recommended for grains and seeds for relevant crops (mentioned in 16.1), seed mixtures and native seeds, and used machinery and equipment. Measures for seeds and grains are considered in detail in Appendix 1. Measures for seed mixtures and native seeds were derived from measures for seeds.

The EWG recommended that measures for grain should apply to all commodities that contain the species specified, i.e. irrespective of whether they are intended for animal feed (incl. bird seeds), human consumption or processing.
The EWG also recommended that new associated crops should be added if *A. tuberculatus* is shown to develop in these crops, and if their seeds or grains may present a risk of contamination with *A. tuberculatus* seeds. The EWG recommended that *A. tuberculatus* should be recommended for regulation as a quarantine pest.

16.1 Measures on individual pathways

Possible pathways *(in order of importance)*	Measures identified
Grains of *Glycine max, Phaseolus vulgaris, Sorghum bicolor* and *Zea mays*	Grains have been produced in a pest-free area for *Amaranthus tuberculatus* established and maintained according to the requirements outlined below Or Grains have been sampled according to ISPM 31 and inspected, and *Amaranthus* seeds have been tested with an approved test, and the grain lot has been found free from *A. tuberculatus* Or Grains have been devitalized according to an appropriate method
Seeds of *Beta vulgaris, Glycine max, Gossypium hirsutum, Medicago sativa, Phaseolus vulgaris, Sorghum bicolor* and *Zea mays*	Seeds have been produced in a pest-free area for *Amaranthus tuberculatus* established and maintained according to the requirements outlined below Or Seeds** have been sampled according to ISPM 31 'Methodologies for sampling of consignments' and inspected, and *Amaranthus* seeds have been tested with an approved test, and the seed lot found free from *A. tuberculatus* *
Seed mixtures and native seeds	Seeds have been produced in a pest-free area for *Amaranthus tuberculatus* established and maintained according to the requirements outlined below Or Seeds have been sampled according to ISPM 31 and inspected, and *Amaranthus* seeds have been tested with an approved test, and the seed lot found free from *A. tuberculatus*
Used agricultural machinery and equipment	ISPM 41 'International movement of used vehicles, machinery and equipment' should be implemented

*Remark: A seed certification scheme includes sampling and testing and is considered to be already covered by this option.
** The seed lot could have been sorted to avoid the presence of the pest.

Requirements for establishing a pest-free area (PFA):

- Detailed surveys and monitoring should be conducted in the area and continued every year. If climatic conditions in the PFA are suitable for the establishment of *A. tuberculatus*, the PFA should not include any area where the species has been reported in the last 10 years.
- Surveys should include high risk locations, such as summer crops, key transportation roads, ports, areas around grain and seed storage facilities etc.
- Where climatic conditions in the PFA are suitable for the establishment of *A. tuberculatus*, there should be restrictions on the movement of the identified pathways for entry (e.g. seeds, grains and used machinery and equipment) into the PFA, and into the area surrounding the PFA, especially the area between the PFA and the closest area of known infestation.

National measures
Early detection is important to identify new occurrences of the species. *Amaranthus tuberculatus* should be monitored and eradicated, contained or controlled where it occurs in the area of potential establishment in the PRA area. In addition, public awareness campaigns to prevent spread from existing populations in countries at high risk are necessary.

16.2 Eradication and containment

Eradication
Eradication measures provided in this section should be promoted where feasible with a planned strategy to include surveillance, containment (see following paragraph), treatment and follow-up measures to assess the success of such actions. As highlighted by EPPO (2012), regional cooperation is essential to promote phytosanitary measures and information exchange in identification and management methods. NPPOs should facilitate collaboration with all sectors to enable early identification including education measures to promote citizen science and linking with universities, land managers and government departments.

Eradication is only considered to be possible for *A. tuberculatus* in case of early detection (newly established populations) of a small population in agricultural productions, or when detected in the natural environment, cargo areas, roadsides and other transportation networks etc. Deep turning of the soil would promote longevity of the seeds and should be avoided. Moreover, seeds on or near the soil surface are more likely to be subject to decay. Eradication measures should include hand weeding (plants being properly disposed) and herbicide treatments (see containment section) to eliminate any escaping plants.

The EWG noted that if the weed is persistent and present in large quantities in an agricultural field, the only feasible eradication method would consist in turning the field into perennial grass for at least 10 years. However, regular surveys would still be required to ensure the area remains free from *A. tuberculatus*.

Eradication may be feasible in some EPPO countries where this species is at an early stage of invasion. It is recommended that member countries eradicate this species where feasible to prevent further spread and impact.

Containment
Unintentional transport of *A. tuberculatus* seeds through the movement of agricultural products and equipment should be avoided. Equipment and machinery should be cleaned to remove the weed seeds before moving to an uninfested area (see ISPM 41: *International movement of used vehicles, machinery and equipment*; FAO, 2017). NPPOs should provide land managers, farmers and stakeholders with identification guides including information on preventive measures and control techniques.

A pro-active and integrated weed management strategy will be required to effectively manage *A. tuberculatus*. General considerations are listed below. It should be noted that in natural environments, management practices should be tailored to the habitat invaded.

Tillage. Heavy tillage, as opposed to light soil disturbance, at the beginning of the season will prepare a proper seedbed for crop planting and eliminate all weeds that have emerged up to this point. Following planting, interrow cultivation can assist to eliminate small seedlings. In general, significant soil disturbance from heavy tillage discourages small-seeded dicots such as *A. tuberculatus*.

Cover crops. Planting agronomic crops into a dense cover-crop can help suppress *A. tuberculatus* germination and emergence. In general, grass cover-crops (such as wheat, rye, barley) can be terminated herbicidally 2-6 weeks prior to summer crop planting. The summer crop can then be planted directly into the terminated cover. Rolling the cover-crop flat and then planting the summer crop in the same direction as rolling will provide greater mulch on soil surface to suppress weed growth. However, if the cover-crop is not dense, the level of weed suppression will be reduced or non-existent.

Crop rotation and management. Planting crops with different life cycles (e.g. winter crops), places *A. tuberculatus* in a disadvantage to germinate and survive. Moreover, this can allow a greater variety of herbicides and other weed management strategies to be used.
Individual crops should be managed to enhance their competitive ability. Depending on crops, this would include row spacing, planting density and planting date. For example, crops with a narrow row spacing can

suppress *A. tuberculatus* growth by shading the soil surface more rapidly. This shading decreases weed germination and suppresses growth of emerged plants.

Surveying and hand weeding. The field should be surveyed, and any remaining weeds should be hand weeded.

Herbicides. Herbicide inputs can be an important component of an integrated weed management plan. However, they must be applied in a timely and proactive manner. Allowing plants to emerge and reach 10-15 cm in height will greatly complicate management with herbicides. Multiple applications of herbicides are necessary to control *A. tuberculatus*.

> **Preemergence.** This refers to soil active herbicides applied after crop planting, but prior to crop or weed emergence. Preemergence herbicides allow the crop to emerge and establish in weed-free conditions. Preemergence herbicides are important because they prevent weed establishment and allow the crop to grow unimpeded. Examples include s-metolachlor, dimethenamid-P, metribuzin, and mesotrione.
>
> **Postemergence.** This refers to herbicides applied after crop and weed emergence. For this application timing to be effective, the herbicide must be applied with sufficient carrier volume to maximize spray coverage on the target weed. It is also important to target *A. tuberculatus* before it exceeds 5 cm in height. Management of larger weeds is considerably more difficult and increases the likelihood of herbicide failure.

Lastly, herbicide resistance to several mechanisms of action is widely documented in *A. tuberculatus*. Prior to developing or planning a herbicide program, analysis of the existing weed population to document the presence or absence of herbicide resistance will be important or essential.

Specific management programs for individual crops as applied in North America are available in e.g. Costea *et al.* (2005), Soltani *et al.* (2009) and Vyn *et al.* (2007).

17. Uncertainty

Main sources of uncertainties in this risk assessment are linked to:

- Effect of different crop systems on the spread and impact in the PRA area compared to the USA (use of herbicide resistant crops, differences in the scale of cultivation areas e.g. for maize and soybean, reliance on herbicides, narrow crop rotation).
- Trade volumes and frequency of movement for some commodities (bird seeds, seed mixtures).
- Uncertainty about additional summer crops *A. tuberculatus* is associated with.
- Role of harvesting equipment and machineries in contaminating other grain commodities before exportation (e.g. rapeseed or winter grains).
- Exact distribution of *A. tuberculatus* in the endangered area of the EPPO region.

18. Remarks

The EWG conducted two PRAs simultaneously on *A. tuberculatus* and *A. palmeri*. Text written in these PRAs have similarities. *Amaranthus tuberculatus* and *A. palmeri* are very similar in their biology, pathways and are both important weeds in North America. However, these species show differences in terms of competitiveness and area of potential establishment in the EPPO region.

The EWG recommended :

- to perform a proper botanical survey in the EPPO region (e.g. during August). This can be performed for *A. palmeri* and *A. tuberculatus* together. If performed on the endangered area identified for *A. tuberculatus*, this would also cover the *A. palmeri* endangered area.
- to take samples where *A. tuberculatus* is present to determine herbicide resistance of the established populations.
- to develop educational materials to help people identifying this species and promote early detection in new areas.

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Appendix 1. Consideration of pest risk management options

The table below summarizes the consideration of possible measures for the pathways '*seeds*' and '*grains (for animal feed mixtures and human consumption)*'. Additional measures were proposed for '*seed mixtures and native seeds* and '*used machinery and equipment*' but are not included in the following table.

For measures, seeds and grains are considered for crops in which *A. tuberculatus* may grow.

When a measure is considered appropriate, it is noted "*yes*", or "*yes, in combination*" if it should be combined with other measures in a systems approach (see after the table). "*No*" indicates that a measure is not considered appropriate. A short justification is included. Elements that are common to several pathways are in bold.

Option	**Grains of *Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays***	**Seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays***
Existing measures in EPPO countries	Partly, see Section 8.	Partly, See section 8.
Options at the place of production		
Visual inspection at place of production	**Yes, in combination* (for measures marked with '*', see after the table).** **The place/site of production when inspected pre-harvest should be free from any *A. tuberculatus* plants.** **Detection by visual inspection is unlikely to be completely effective at the place of production in plants used to produce grains or seeds and needs to be used within a systems approach.**	Yes, in combination* As for grains
Testing at place of production	**No** **Testing would only allow to confirm the identity of *Amaranthus* spp. observed at or around the place of production based on visual examination.**	No As for grains
Treatment of crop	**Yes, in combination*** **No weed management strategy is considered to be 100% effective against *A. tuberculatus*.**	Yes, in combination* As for grains
Resistant cultivars	**No, not relevant for invasive alien plants (IAPs)**	No, not relevant for invasive alien plants (IAPs) As for grains.
Growing the crop in glasshouses/ screenhouses	Not relevant for grain production.	No This option could only very rarely be used for some of the listed

Option	Grains of *Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*	Seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*
		species (e.g. for maintenance and production of maize parent lines or production of parent seed stocks and has therefore not been kept as an option. Such material for scientific or selection purpose may be imported under a post-entry quarantine bilateral agreement between the importing and the exporting country. Growing the crop in glasshouses alone would not prevent the risk of entry in the glasshouse with the planted seeds themselves.
Specified age/size of plant, growth stage or time of year of harvest	**No,** ***A. tuberculatus* may be present and produce seeds during the entire growing season of the crop.**	No As for grains.
Produced in a certification scheme	No, not relevant for grains	Yes. The seeds should be free from *A. tuberculatus* seeds, based on a sampling conducted in accordance with ISPM 31. A purity check will be performed on the sample to guarantee the absence of *A. tuberculatus* seeds. In case *Amaranthus* seeds are present, these seeds should be tested, and the seed lot found free from *A. tuberculatus*.
Pest free production site	**No** **The EWG considered that due to the high seed production, the longevity of the soil seed bank and the spread potential, a pest-free production site is not a feasible option in an area where *A. tuberculatus* is present.**	No As for grains
Pest free place of production	**No, as for pest free production sites**	No As for grains
Pest-free area	**Yes** • **To establish and maintain the PFA, detailed surveys and monitoring should be conducted in the area and continued every year. If climatic conditions in the PFA are suitable for the establishment of *A. tuberculatus*, the PFA should not include any area where the species has been reported in the last 10 years.** • **Surveys should include high risk locations, such as summer crops, key transportation roads, ports, areas around grain and seed storage facilities etc.**	Yes, As for grains.

Option	**Grains of *Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays***	**Seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays***
	• **Where climatic conditions in the PFA are suitable for the establishment of *A. tuberculatus*, there should be restrictions on the movement of the identified pathways for entry (e.g. seeds, grains) into the PFA, and into the area surrounding the PFA, especially the area between the PFA and the closest area of known infestation.**	
Options after harvest, at pre-clearance or during transport		
Treatment of the consignment: sorting	**Yes, in combination*** **Automatic sorting (e.g. optical, density, with vibrating mesh, rotary drum, with aspirator, etc.) can be performed, especially in grain and seeds that differ significantly in size, weight and/or colour.** **The efficiency of screening depends on the sorting methodology used (e.g. type of screens) and the seed size of grain and weeds (Australia biosecurity, 2002).**	Yes, in combination* As for grains.
Treatment of the consignment: devitalization	Yes When sorting is not feasible, devitalization may be performed such as described in Australia-Biosecurity (2002) for maize and Blythman & Samson (2019) for bird seeds. In particular, Australia-Biosecurity (2002) reported that steam treatment at 95-100°C for 12-15 minutes killed several weed species including *Amaranthus* spp. Therefore, steam heat treatment of imported maize would manage the risk effectively, particularly if the treatment could be conducted at the port of entry or just prior to export, minimising the opportunities for post-treatment re-contamination.	No Devitalization is not possible for seeds
Visual inspection of consignment and confirmation by testing	**Yes** **Tests allow detection of the weed seeds in mixed grains/seeds. After having performed a purity/noxious weed examination, *Amaranthus* seeds, either individually or in pools from the same lot, may be submitted for testing. The sampling of the consignment should be conducted in accordance with ISPM 31. Remark: because of the size of *A. tuberculatus* seeds, they will not be equally distributed in the seed/grain commodity**	Yes As for grain.

Option	Grains of *Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*	Seeds of *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*
	Remark: this may not be cost-effective for some grain commodities.	
Options that can be implemented after entry of consignments		
Post-entry quarantine	Not relevant for grain.	Not relevant for seed.
Limited distribution of consignments in time and/or space or limited use	Not relevant The use of grains cannot be limited to reduce the probability of introduction: processing grain could be partially or totally destructive but seeds of *A. tuberculatus* may be spread during storage and transportation.	Not relevant. The use of seeds cannot be limited to reduce the probability of entry.
Only surveillance and eradication in the importing country	**No.** **Eradication is difficult.**	No. As for grains

*The EWG considered whether the measures identified above as 'Yes in combination' (listed below) could be combined to achieve a suitable level of security. This was not possible for these commodities. It is considered that there is too much variability in the application of the treatment methods of the crop and the sorting to allow a combination of these measures.

Grains of *Glycine max*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*	**Seeds of** *Beta vulgaris*, *Glycine max*, *Gossypium hirsutum*, *Medicago sativa*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Zea mays*
Visual inspection at place of production	Visual inspection at place of production
Treatment of crop	Treatment of crop
Treatment of consignment: sorting	Treatment of consignment: sorting

Appendix 2. Relevant illustrative pictures (for information)

Public domain https://commons.wikimedia.org/wiki/File:Amaranthus_tuberculatus_drawing.jpg

Amaranthus tuberculatus seed and capsule (Government of Canada)

Amaranthus palmeri seedling (left) with *A. tuberculatus* (right) (EPPO Global database)

Amaranthus tuberculatus Surviving post-emergence application due to poor timing of application (EPPO Global database)

Appendix 3 Projection of climate suitability for *A. tuberculatus* establishment in the EPPO region

Aim

To project the climatic suitability for potential establishment of *Amaranthus tuberculatus* in Europe and the Mediterranean region, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF), Integrated Digitized Biocollections (iDigBio), USGS Biodiversity Information Serving Our Nation (BISON), Acta Plantarum and additional literature records (Alessandrini *et al.*, 2012; Barberis *et al.*, 2013; Bertolli & Prosser, 2014; Gullón & Verloove, 2015). With the EWG, the records were scrutinized to remove any considered too old (<1970) or of dubious quality. This included removing records from the countries in which the species is classified as casual. Records were classified as native or non-native based on published distributions at US state level (Plants of the World Online, BONAP, CABI ISC).

The records were gridded at a 0.25 x 0.25 degree resolution for modelling (Figure 1a). This resulted in 528 grid cells containing records of *A. tuberculatus* (Figure 1a), which is a sufficient number for distribution modelling.

Based on the life history requirements of *A. tuberculatus* and likely limiting factors for establishment in Europe, the following predictor variables were assembled on the same grid:

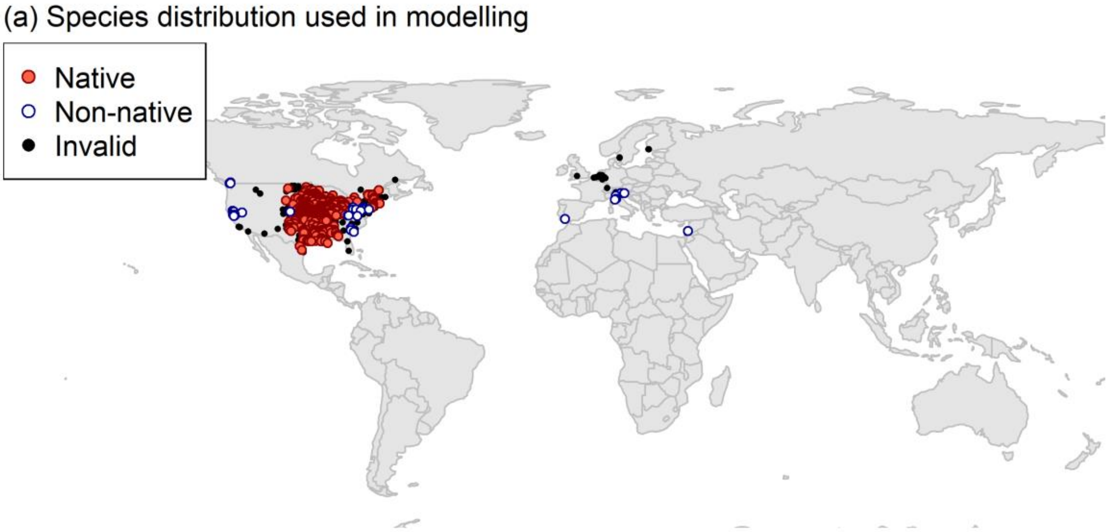
- <u>Mean minimum daily temperature of the coldest month</u> (Bio6 °C) from WorldClim v2 (Fick & Hijmans, 2017). Seed germination of *A. tuberculatus* is benefitted by cold stratification at 4 °C (Leon *et al.*, 2007).
- <u>Mean temperature of the warmest quarter</u> (Bio10 °C) from WorldClim v2 (Fick & Hijmans, 2017). In *A. tuberculatus* seed germination and biomass accumulation are limited by low temperature with very little of either below 15 °C and optimal temperatures between 20-30 °C (Guo & Al-Khatib, 2003; Steckel *et al.*, 2004).
- <u>Potential Evapotranspiration</u> (PET mm yr^{-1}) estimated using monthly WorldClim v2 temperatures (Fick & Hijmans, 2017) following Zomer *et al* (2008). This is an alternative measure of solar energy available for growth, more strongly linked to latitude than Bio10.
- <u>Climatic moisture index (CMI, ln+1 transformed)</u> calculated as annual precipitation (Bio12 from Worldclim v2; Fick & Hijmans, 2017) divided by PET and reflecting moisture availability for plants. As a hygrophyte to mesophyte, *A. tuberculatus* may avoid very arid areas (Costea *et al.*, 2005).
- <u>Urban cover</u> derived from GlobCover 2009 v2.3 urban class ("Artificial surfaces and associated areas (Urban areas >50%)") (Bontemps *et al.*, 2011). As a species often dispersed by humans (Costea *et al.*, 2005), *Amaranthus tuberculatus* may favour urban areas.
- <u>Cropland cover</u> derived from GlobCover 2009 v2.3 cropland classes ("Post-flooding or irrigated croplands (or aquatic)", "Rainfed crops", "Mosaic cropland (50-70%) / vegetation (grassland/shrubland/forest) (20-50%)" and "Mosaic vegetation (grassland/shrubland/forest) (50-70%) / cropland (20-50%)") (Bontemps *et al.*, 2011). *Amaranthus tuberculatus* is a successful weed in croplands (Costea *et al.*, 2005).
- <u>Preferred crop area</u> (km^2) derived from global harvested areas of alfalfa, bean, cotton, green bean, maize, rice, sorghum, soybean and string bean, sugar beet and sunflower (Monfreda *et al.*, 2008).
- <u>River length (km)</u> calculated from the hydroRIVER database (Lehner & Grill, 2013). Riverbanks are a preferred habitat of *A. tuberculatus* (Costea *et al.*, 2005).

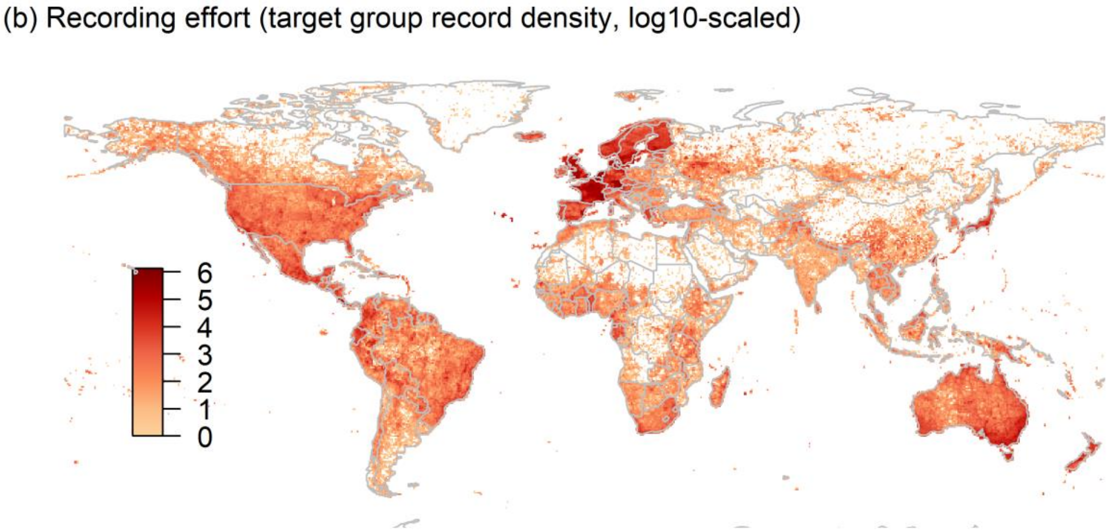
To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathway (RCP) 4.5 and 8.5 were also obtained. For both scenarios, the above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim v1 baseline.

RCP 4.5 is a moderate climate change scenario in which CO_2 concentrations increase to approximately 575 ppm by the 2070s and then stabilise, resulting in a modelled global temperature rise of 1.8 °C by 2100. RCP8.5 is the most extreme of the RCP scenarios, and may therefore represent the worst case scenario for reasonably anticipated climate change. In RCP8.5 atmospheric CO_2 concentrations increase to approximately 850 ppm by the 2070s, resulting in a modelled global mean temperature rise of 3.7 °C by 2100.

Finally, the recording density of vascular plants (phylum Tracheophyta) on GBIF was obtained as a proxy for spatial recording effort bias (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Amaranthus tuberculatus*, showing the native and non-native records used in modelling as well as the invalid records not used in the modelling (old, undated, inaccurate or casual). (b) A proxy for recording effort – the number of post-1970 vascular plant records held by the Global Biodiversity Information Facility, displayed on a log_{10} scale.





Species distribution model

The modelling followed a recent modification of standard presence-background (presence-only) ensemble distribution modelling for emerging invasive non-native species (Chapman *et al.*, 2019). This accounts for dispersal constraints on non-equilibrium invasive species' distributions (Elith *et al.*, 2010) by excluding locations suitable for the species but where it has not been able to disperse to.

To do this, background samples (pseudo-absences) were sampled from two distinct background regions:

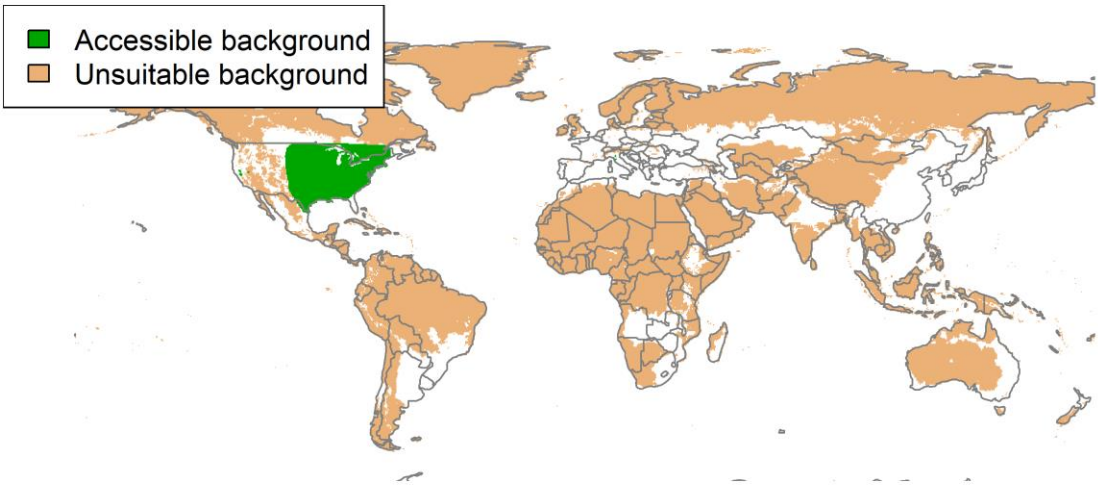
- An <u>accessible background</u> includes places close to *A. tuberculatus* populations, in which the species is likely to have had sufficient time to disperse and sample the range of environments. The accessible background was defined as a 200 km buffer around the native range (minimum convex polygon bounding native occurrences) and a 30 km buffer around non-native occurrences (capturing a 4-cell neighbourhood of the non-native occurrences). Sampling was more restrictive from the invaded range to account for stronger dispersal constraint over a shorter residence time. Alternative buffer radii were also tested but did not substantively affect the model projections.
- An <u>unsuitable background</u> includes places expected to be physiologically unsuitable for the species, so that absence will be irrespective of dispersal constraints. No specific ecophysiological information was available so extreme values of the predictors at the species occurrences was used to define unsuitability as:

- Mean temperature of the warmest quarter (Bio10) < 15 °C, a minimum temperature for *A. tuberculatus* germination and growth (Guo & Al-Khatib, 2003; Steckel *et al.*, 2004); OR
    - Minimum temperature of the coldest month (Bio6) > 13 °C, presumed too warm for seed stratification; OR
    - PET < 600, presumed too low energy for growth; OR
    - Climatic moisture index < 0.25, presumed too dry for occurrence.

Two occurrences (0.37%) fell in the unsuitable background.
For modelling, five random background samples were obtained as follows:

- From the accessible background 528 samples were drawn, which is the same number as the occurrences. Sampling was performed with realistic recording bias using the target group approach (Phillips, 2009) in which sampling was weighted by GBIF recording density (Figure 1b). Taking the same number of background samples as occurrences ensured the background sample had the same level of bias as the data.
- From the unsuitable background 5000 simple random samples were taken. Sampling was not adjusted for recording biases as we are confident of absence from these regions.

Figure 2. The background regions from which 'pseudo-absences' were sampled for modelling. (a) The accessible background is assumed to represent the range of environments the species has had chance to sample. (b) The unsuitable background is assumed to be environmentally unsuitable for the species.



Using these data, a presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7 (Thuiller *et al.*, 2009, 2016). Each dataset (presences and the five individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings (except where specified below) and rescaled using logistic regression:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per effect.
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent (Phillips *et al.*, 2008)

Prevalence weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure. Model predictive performance was assessed by calculating the Area Under the Receiver-Operator Curve (AUC) for model predictions on the evaluation data, which were reserved from model fitting. AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected pseudo-absence.

An ensemble model was created by rejecting poorly performing algorithms and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability.
Global model projections were made for the current climate and for the two climate change scenarios, avoiding model extrapolation beyond the ranges of the input variables. The optimal threshold for partitioning the ensemble predictions into suitable and unsuitable regions was determined using the 'minRocDist' method (Manel *et al.*, 2001).
Limiting factor maps were produced following Elith *et al*. (2010). Projections were made separately with each individual variable fixed at a near-optimal value (median values at the occurrence grid cells). Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results
The ensemble model suggested that suitability for *A. tuberculatus* at the global scale and resolution of the model was most strongly limited by low summer temperature (Bio10), high winter temperature (Bio6) and low moisture (CMI) and lack of preferred crops (Table 1, Figure 3). The model fitted weaker effects of PET, urban and croplands cover and rivers.

Global projection of the ensemble model in current climatic conditions indicates that nearly all native and known invaded records fell within regions predicted to have high suitability (Figure 4).
Across Europe and the Mediterranean region, the model predicts a climatically suitable range in countries bordering the northern and eastern Mediterranean Sea, as well as in the Pannonian Plain, countries bordering the Black Sea and in central Asia. Some marginally suitable areas are predicted in North Africa (Figure 5). The model suggests the main limiting factor in unsuitable parts of northern Europe is low summer temperature (Bio10), while drought stress (low CMI) was the strongest limiting factor in north Africa and the Middle East (Figure 7).
Predictions of the model for the 2070s, under the moderate RCP4.5 and extreme RCP8.5 climate change scenarios, suggest large increases in suitability in Europe driven by warmer summers (Figures 7 and 8). The climatically suitable range may extend northwards to the southern Baltic coast, assuming no change in land use. Southern parts of the currently suitable region become less unsuitable as a result of drier conditions and warmer winters (Figures 7 and 8).
These results are reflected in the suitability of different European Biogeographical Regions (Bundesamt fur Naturschutz (BfN), 2003) (Figure 9). Regions highly suitable under current and future climate scenarios are the Pannonian and Mediterranean. Under climate change, suitability of the Mediterranean slightls reduces, while regions such as Continental, Black Sea and Steppic become much more suitable.
In terms of EPPO member states, Hungary, Croatia, Albania, Bulgaria, Serbia, Greece, Moldova, Italy, Portugal and Romania are all predicted to be >50% suitable currently (Table 2). Under the most extreme warming scenario, those countries remain highly suitable except for Portugal. In addition, the following countries are also predicted to become >50% suitable: Montenegro, Macedonia, Bosnia and Herzegovina, Ukraine, Slovenia, Czech Republic, Slovakia, Poland, France, Georgia and Belarus (Table 2).

Table 1. Summary of the cross-validation predictive performance (AUC) and variable importances of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to five different background samples of the data.

Algorithm	**AUC**	**In the ensemble**	**Variable importance**							
			Minimum temperature of coldest month (Bio6)	**Mean temperature of warmest quarter (Bio10)**	**Potential Evapotranspiration (PET)**	**Climatic moisture index (CMI)**	**Cropland cover**	**Urban cover**	**Preferred crop area**	**River length**
GLM	0.9778	yes	35%	26%	18%	17%	2%	0%	1%	1%
GAM	0.9772	yes	25%	41%	12%	18%	2%	0%	0%	2%
ANN	0.9730	no	17%	25%	23%	7%	0%	0%	25%	2%
GBM	0.9808	yes	25%	30%	2%	10%	1%	4%	27%	0%
MARS	0.9786	yes	30%	35%	10%	21%	0%	0%	4%	0%
RF	0.9770	yes	24%	18%	6%	6%	5%	12%	25%	3%
Maxent	0.9800	yes	25%	34%	6%	14%	6%	2%	12%	1%
Ensemble	0.9812		27%	31%	9%	14%	3%	3%	12%	1%

Figure 3. Partial response plots from the individual algorithms and ensemble model (thick black lines), ordered from most to least important. In each plot, other model variables are held at their median value in the training data. Variable codes: Bio10 = mean temperature of warmest quarter (°C); Bio6 = minimum temperature of coldest month (°C); CMI = climatic moisture index (ln+1); pref_crops = area of preferred crops (km^2); PET = potential evapotranspiration (mm yr^{-1}); urban = proportion cover of urban areas; crops = proportion cover of cropland; rivers = river length (km).

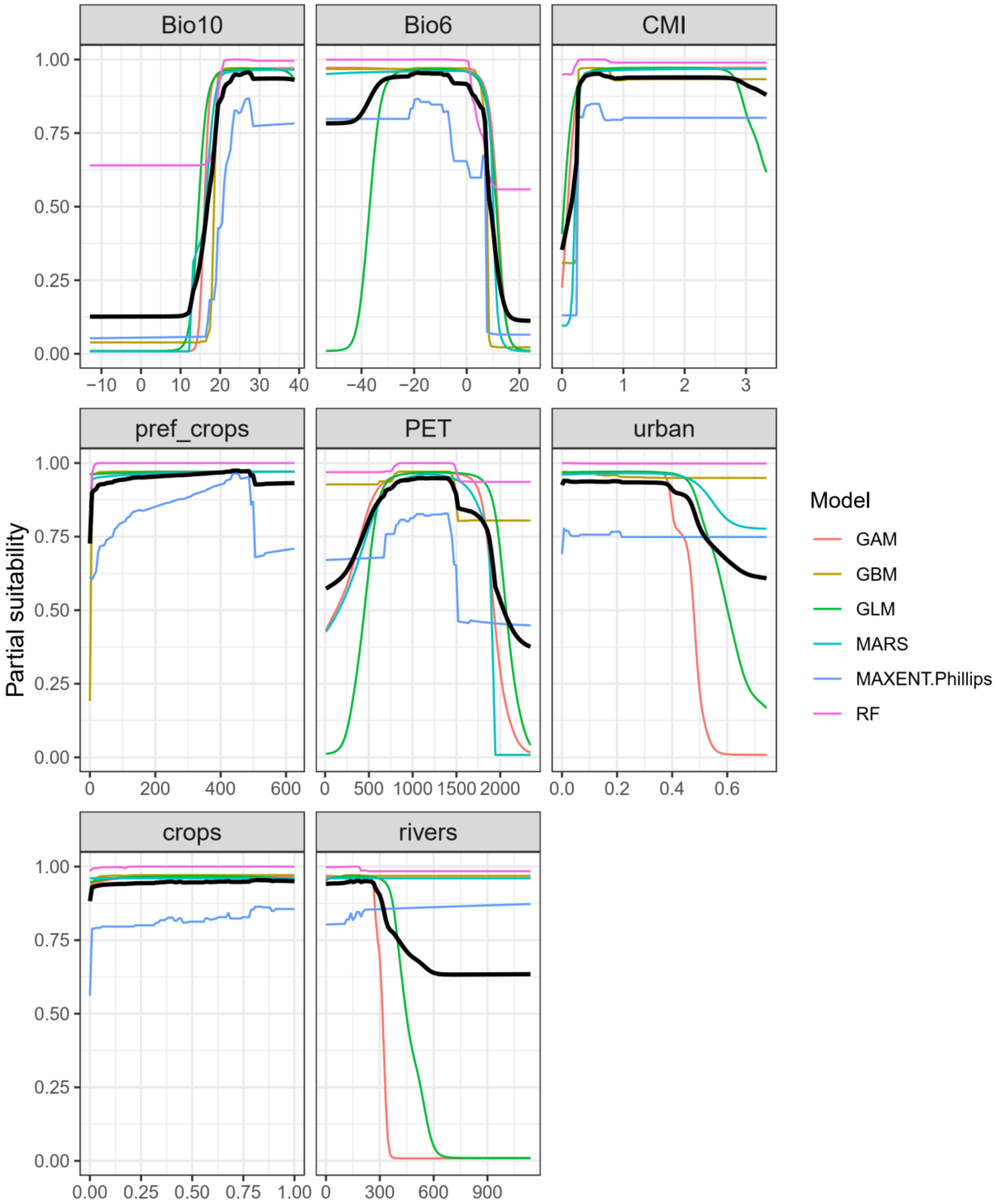


Figure 4. (a) Projected global suitability for *Amaranthus tuberculatus* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Red shading indicates suitability, according to the selected threshold. White areas have climatic conditions outside the range of the training data so were excluded from the projection. (b) Uncertainty in the suitability projections, expressed as the standard deviation of projections from different algorithms in the ensemble model.

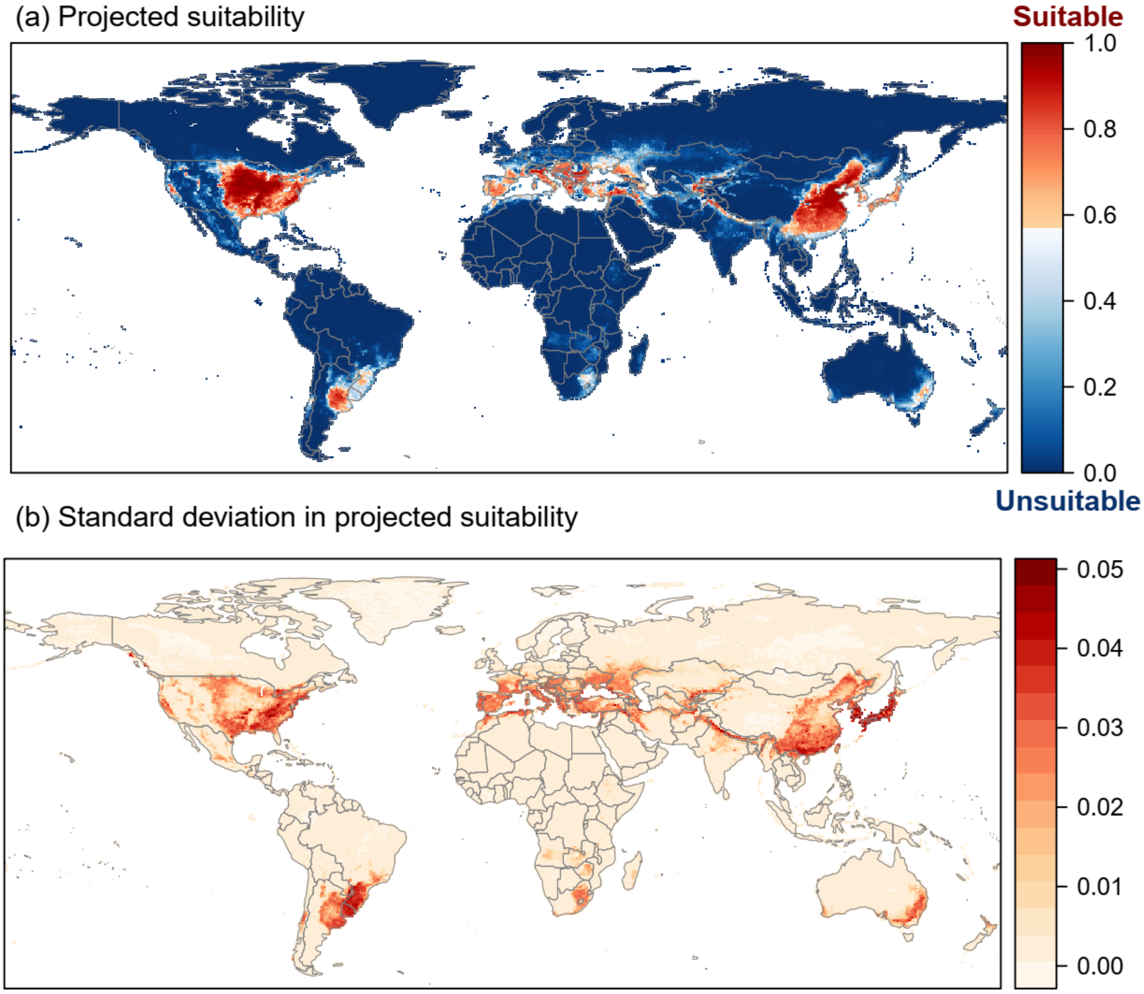


Figure 5. Projected current suitability for *Amaranthus tuberculatus* establishment in Europe and the Mediterranean region. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

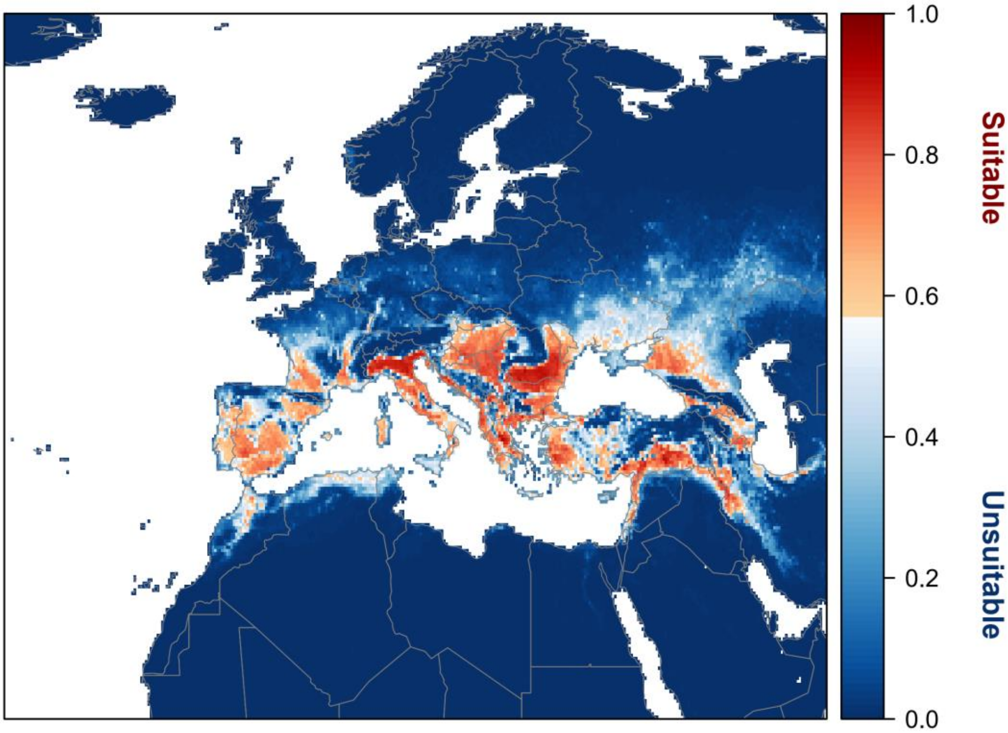


Figure 6. Limiting factor map for *Amaranthus tuberculatus* in Europe and the Mediterranean region in the current climate. Colours show the variable most strongly limiting suitability.

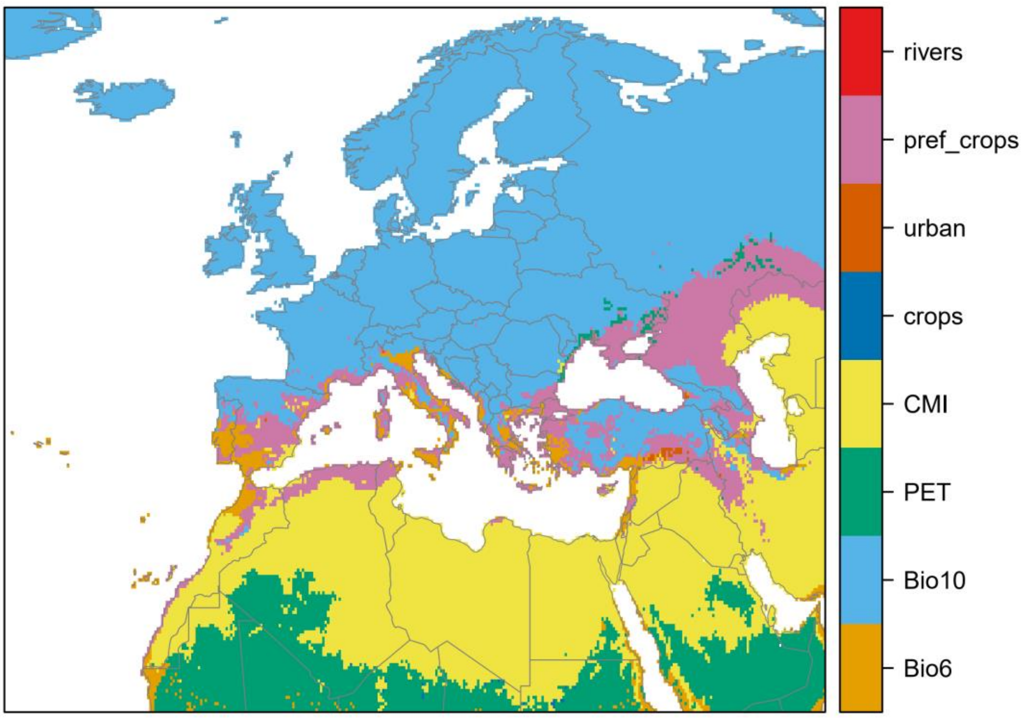


Figure 7. Projected suitability for *Amaranthus tuberculatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, as Figure 5.

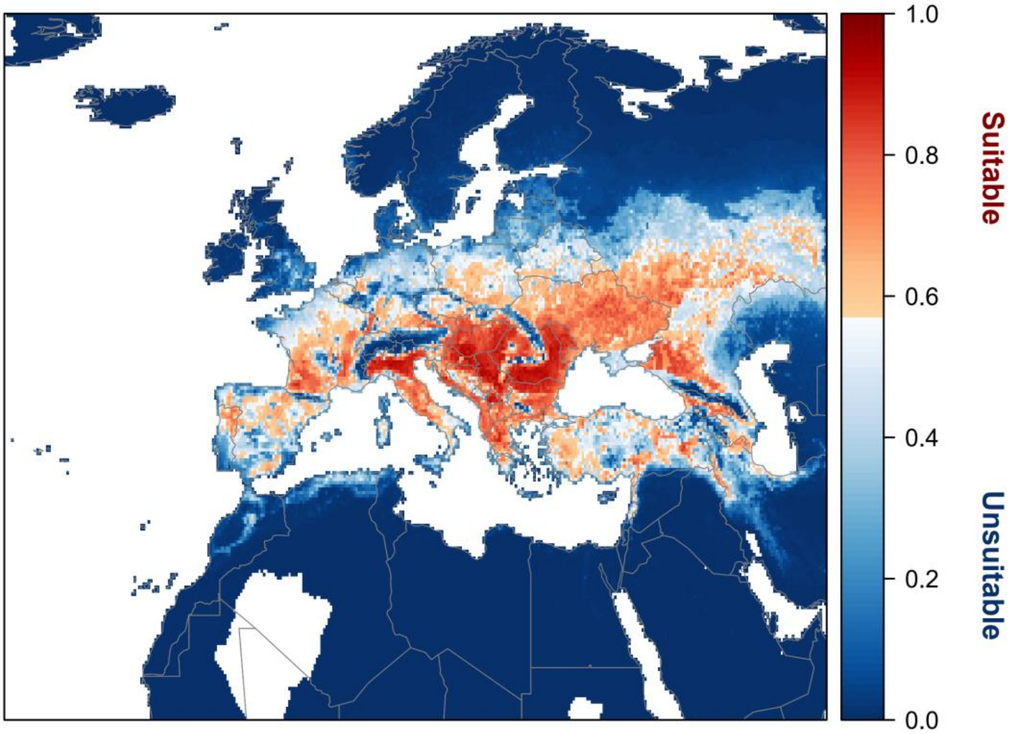


Figure 8. Projected suitability for *Amaranthus tuberculatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP8.5, as Figure 5.

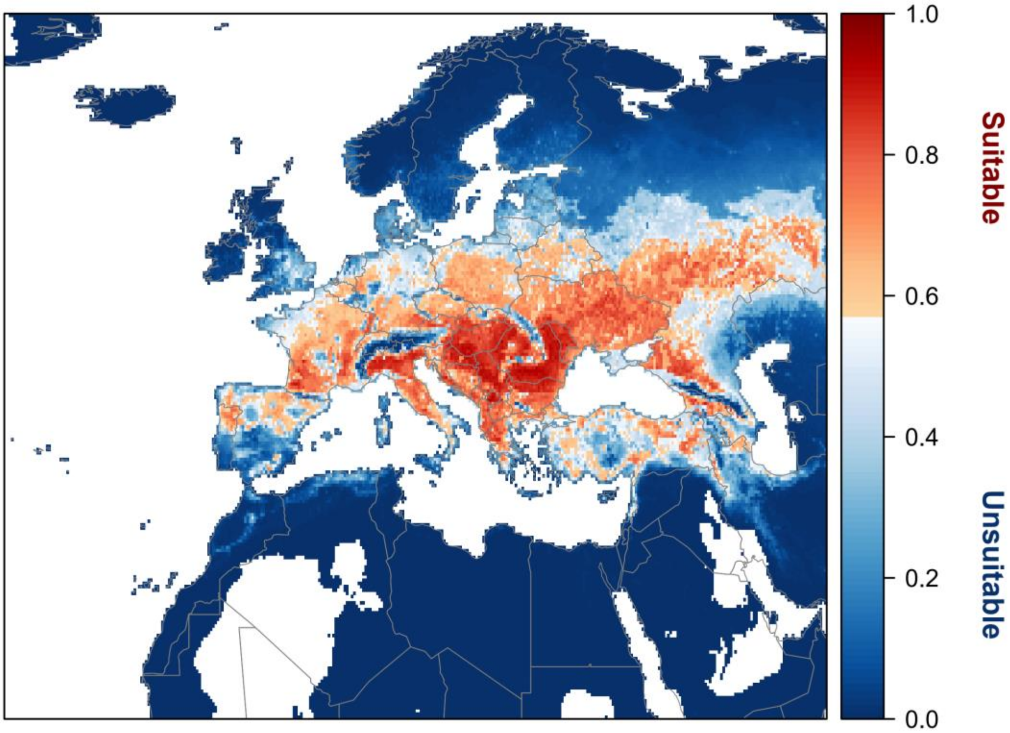
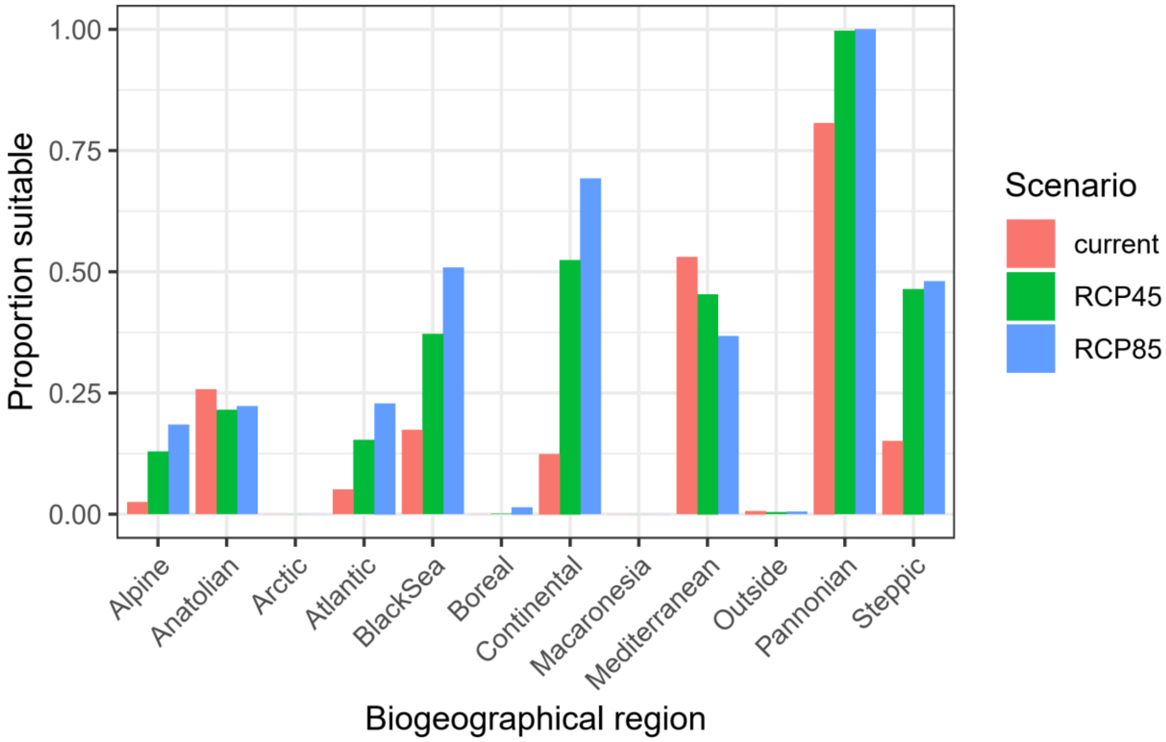


Figure 9. Variation in projected suitability among Biogeographical regions of Europe (Bundesamt fur Naturschutz (BfN), 2003). Bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under emissions scenarios RCP4.5 and RCP8.5. The coverage of each region is shown in the map below.



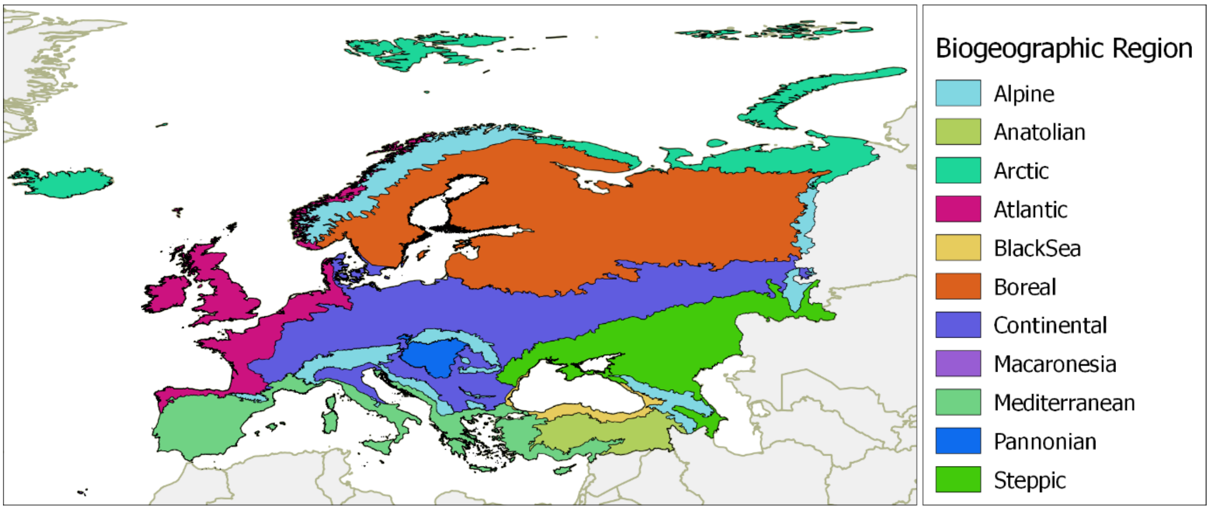


Table 2. Projected % suitability among EPPO member countries, sorted from high to low. Values are the % of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under emissions scenarios RCP4.5 and RCP8.5.

EPPO country (ISO3)	Current	RCP4.5	RCP8.5	EPPO country (ISO3)	Current	RCP4.5	RCP8.5
HUN	82	99	100	KAZ	1	1	2
HRV	66	94	95	RUS	0	3	4
ALB	65	94	92	DZA	0	0	0
BGR	65	86	88	POL	0	34	65
SRB	62	95	99	CZE	0	31	75
GRC	62	65	54	DEU	0	19	39
MDA	62	100	100	CHE	0	16	33
ITA	53	56	56	BLR	0	15	53
PRT	53	33	33	BEL	0	13	43
ROU	52	82	88	NLD	0	4	17
ESP	45	30	23	LTU	0	0	6
MKD	45	95	98	CYP	0	0	0
BIH	36	75	96	DNK	0	0	0
AZE	35	39	23	EST	0	0	0
GEO	34	47	54	FIN	0	0	0
TUR	33	33	30	GBR	0	0	0
MNE	22	61	100	GGY	0	0	0
SVK	16	59	73	IRL	0	0	0
FRA	14	48	62	JEY	0	0	0
SVN	10	62	77	JOR	0	0	0
UZB	8	5	5	LUX	0	0	0
KGZ	8	11	15	LVA	0	0	0
ISR	6	0	0	MLT	0	0	0
UKR	6	87	90	NOR	0	0	0
MAR	3	0	0	SWE	0	0	0
AUT	1	38	45	TUN	0	0	0

Caveats and uncertainties

Modelling the potential distributions of range-expanding species is always difficult and uncertain. In this case study, uncertainty arises because:

- The models were constructed using convenient climate and habitat layers, which may not be the most appropriate for *A. tuberculatus*. Specific predictors layers capturing requirements for different stages of the life cycle (e.g. for germination in spring or seed ripening in late summer) may have improved the predictions.
- The selection of the background sample was weighted by the density of vascular plant records on the Global Biodiversity Information Facility (GBIF) to reduce spatial recording bias. While this is preferable to not accounting for recording bias at all, a number of factors mean this may not be the perfect null model for species recording, especially because additional data sources to GBIF were used.

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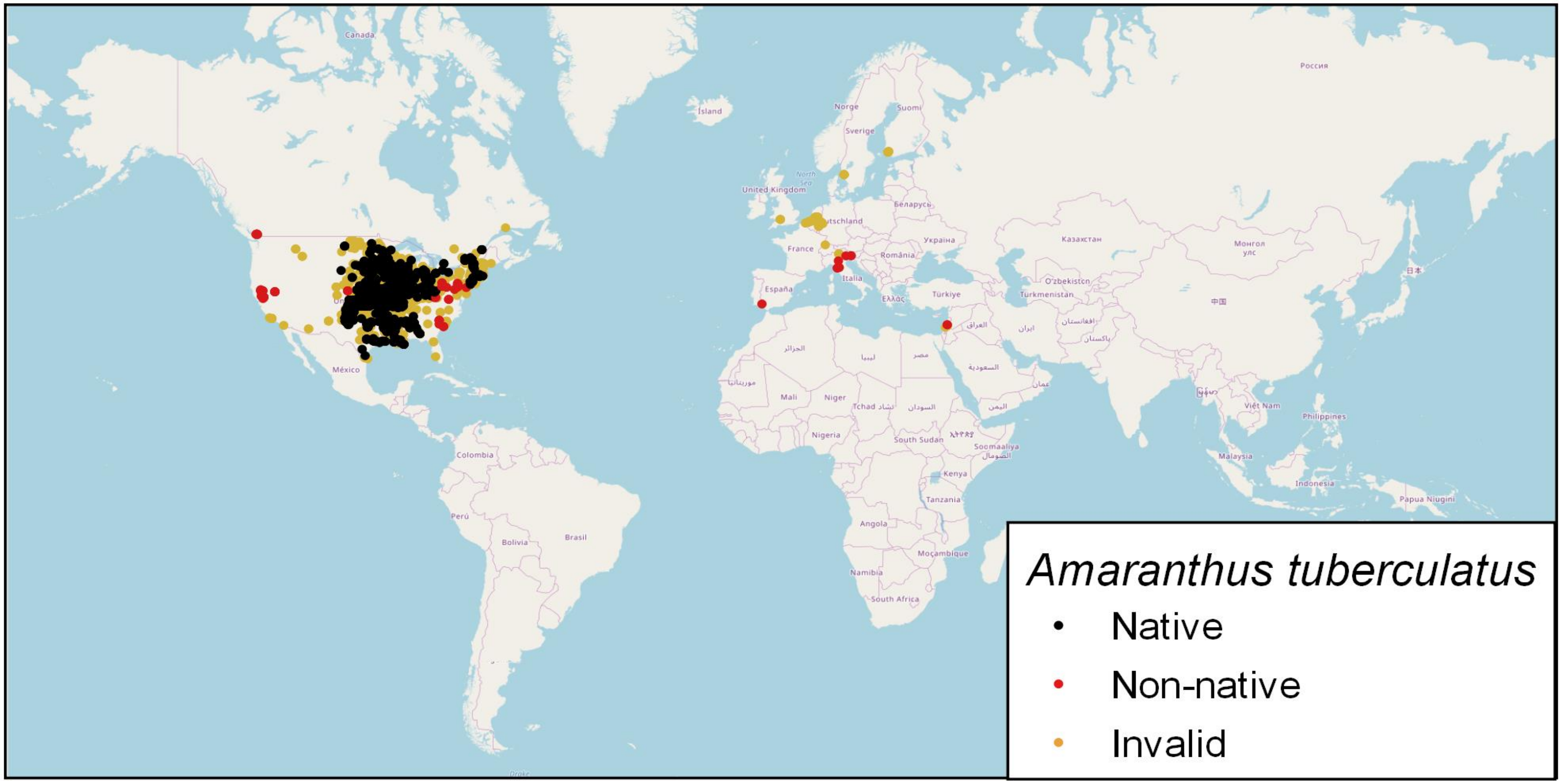
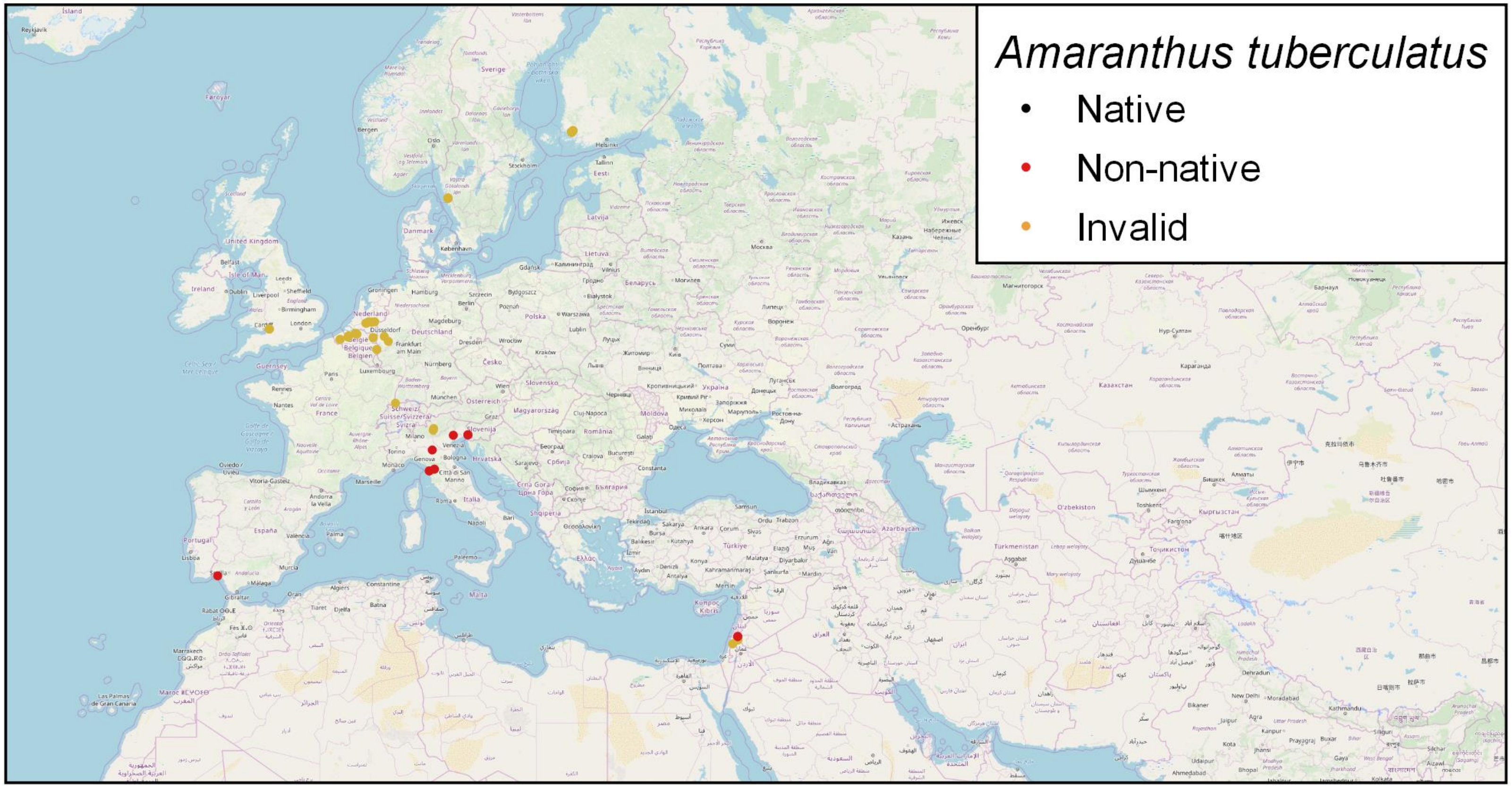
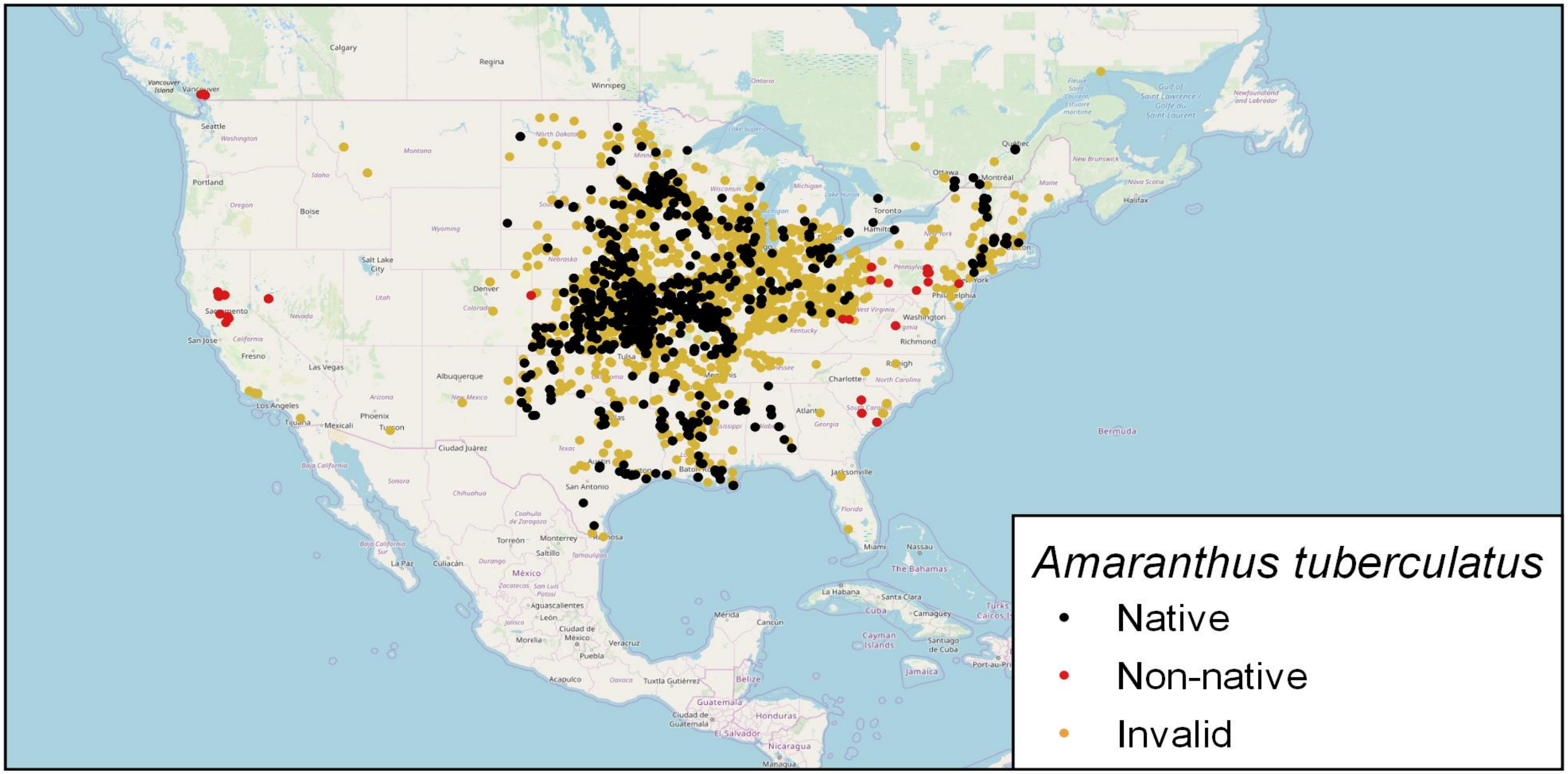
Appendix 4 Distribution of *Amaranthus tuberculatus* data used for the modelling



Global data

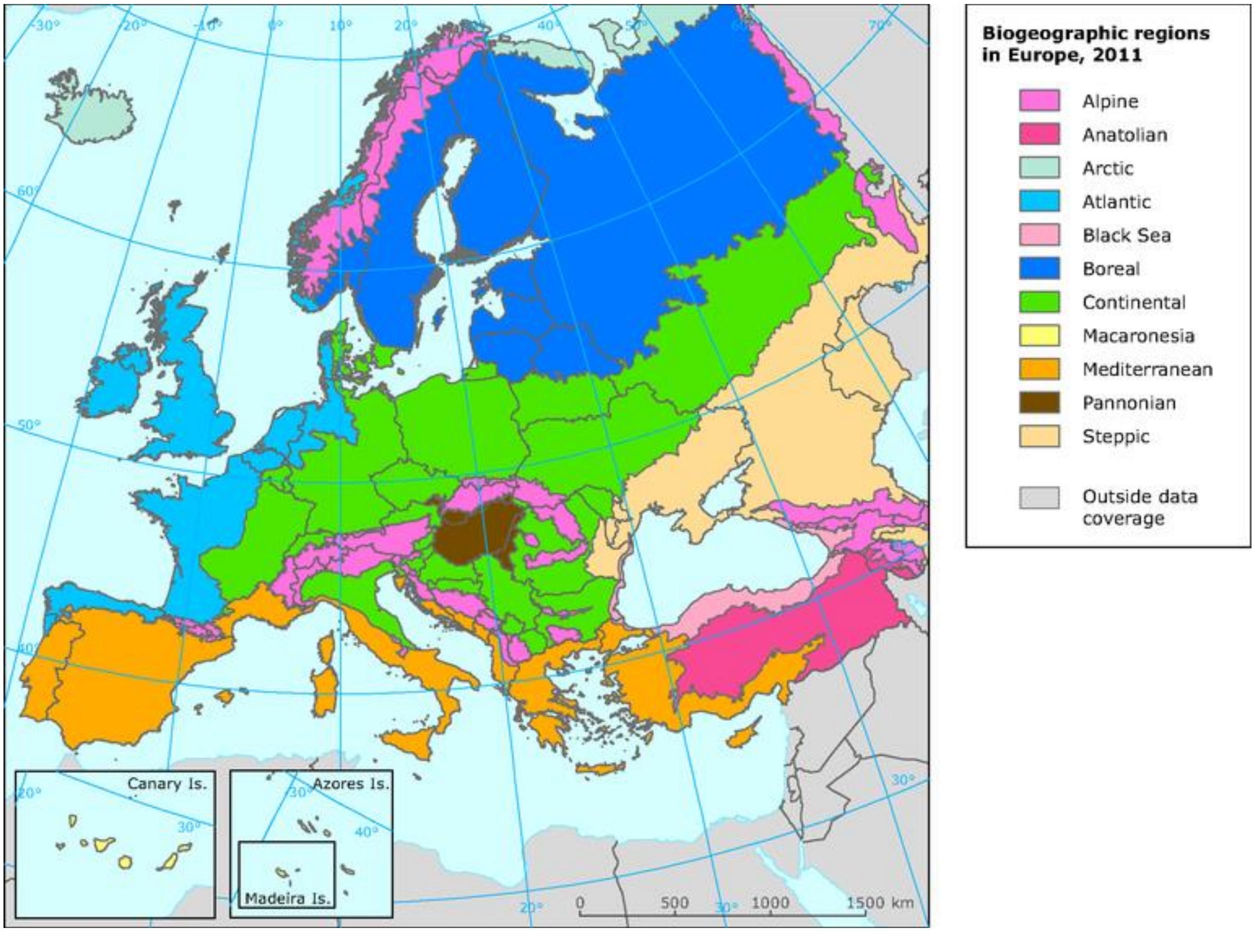


EPPO region data



North American data

Appendix 5 Biogeographical regions in Europe



Appendix 6 Grain imports from USA into the EPPO region

Table 1. Imports of soybean grain into EPPO countries from the USA from 2015-2018. The following commodities have been combined (Soybean (other) HS code: 1201900095), Soybean seeds of a kind used as oil stock HS code: 1201900005). The data for 2018 is from Jan-Nov. Figures detail in metric tonnes per year.

Country	2015	2016	2017	2018
Azerbaijan	0	0	0	10493
Finland	333	234	273	272
France	104165	272466	64900	182732
Germany	2191796	1308642.3	1314686	901860
Greece	0	17000	14114	57038
Ireland	0	2600	4637	0
Israel	73	74141	79454	119956.1
Italy	50089.7	201452	75523	881304
Lithuania	0	0	0	2.9
Morocco	109222	66092	55722	39785
Netherlands	1119010	1909165	2045877	3784707.2
Poland	1453	0	105	30000
Portugal	197565	57812	123156	472551
Romania	67822	0	0	113477
Russia	510507	155547	0	0
Spain	1041898	895232	607995	1812908.1
Tunisia	152036	362771	221094	448182
Turkey	509695.8	157369	368627	240078
Ukraine	20	232	120	47
United Kingdom	200185	229897	100	326894.5

Table 2. Imports of maize grain into EPPO countries from the USA from 2015-2018. The following commodities have been combined (HS Code: 1005902045 No. 4 corn X SD, HS code: 1005904055 corn white EX SD, HS code: 1005904065 corn NES, 1005902020 No. 1 Corn EX SD, HS Code: 1005902035, No. 3 corn, EX SD). The data for 2018 is from Jan-Nov. Figures detail in metric tonnes per year.

Country	2015	2016	2017	2018
Algeria	238846	678575	75373	47627
Austria	0	3396	0	0
France	0	799	19	0
Germany	0	743	343	0
Greece	0	0	0	81
Ireland	61322	280515	140149	111
Israel	16180	387811	107459	814810
Italy	0	19	27816	29502
Jordan	80441	61778	155984	38
Lithuania	0	0	0	42
Morocco	268286	772927	575272	822679
Netherlands	0	84457	210197	439800
Norway	0	0	0	47
Poland	0	0	0	51
Portugal	152089	109026	118335	227473
Romania	0	0	0	0
Russia	1313	0	0	0
Spain	66299	85079	185613	1167083
Tunisia	38189	177691	20000	451707
Turkey	13199	2679	80	585
Ukraine	0	0	42	0
United Kingdom	293	43851	434	19888

Appendix 7 Imports of seed of crops that may be contaminated by *A. tuberculatus* from USA into the EPPO region (Data from FAO Stats)

Table 1. Maize seed for planting imports into EPPO countries from the USA from 2015-2018. The following commodities have been combined (Corn SD Other (HS code: 1005100090), Corn SD Yellow (HS code 1005100010), Sweet Corn SD (HS code: 712908550)). The data for 2018 is from Jan-Nov. Figures detail in metric tonnes per year.

Country	2015	2016	2017	2018
Albania	0	40.2	0	18.6
Algeria	0	5.9	119.9	0
Austria	52.6	67	0	221
Belgium	0.1	19.6	105.5	111.8
Croatia	2.4	3	0	0.2
Cyprus	0	0	4.5	54.3
Denmark	0	0.2	0	0.7
Finland	0.9	0	0	0
France	2848.4	2586.5	3269.5	2028.7
Germany	77	109.7	126.7	139.4
Greece	44.1	164.3	22.8	99.1
Hungary	155.2	103.4	86.6	84.5
Ireland	4.6	0	0	0
Israel	35	52.3	87.4	66.5
Italy	674.1	1123.1	693.3	485.5
Jordan	91.2	18.9	26.8	24.4
Kazakhstan	0	0.9	7	102
Kyrgyzstan	0.4	1.1	0.4	0.1
Morocco	0	0	0	2.5
Netherlands	844.2	372.5	232	308.5
Poland	0	0	40	0
Portugal	0	15	11.4	1.1
Romania	5.4	0.7	0	2.1
Russia	0	0	0	5.8
Serbia	1.6	1.2	2.2	4.2
Spain	2059.5	407	132.6	62.1
Switzerland	1.8	9.1	0	0
Turkey	236.2	133.9	103.2	72.2
Ukraine	18.3	14.3	29.2	152.2
United Kingdom	294.2	216.1	354.2	380
Uzbekistan, Republic of	3.6	5.8	6.9	1.3

Table 2. Sorghum seed for planting imports into EPPO countries from the USA from 2015-2018. The following commodities have been combined (Sorghum seed (HS code: 1007100000) and Sorghum/Sudan SD (HS code: 1209299150)). The data for 2018 is from Jan-Nov. Figures detail in metric tonnes per year.

Country	2015	2016	2017	2018
Algeria	641.9	1092.1	599.3	293.6
Austria	4.7	0	0.8	0
Cyprus	14	0	0	0
France	1785.3	215.1	329.2	377.2
Germany	279.9	536.9	102.3	47.5
Greece	119	118	118	72
Hungary	236.6	555.2	287.4	0
Israel	38.8	0.8	0	0
Italy	1513.4	417.7	1021.2	1379
Jordan	0	0	3	0
Kazakhstan	0	0	25.2	0
Morocco	79.1	239.9	197.5	38.8
Netherlands	0	4.1	359.5	60
Poland	0	0	20	32.5
Portugal	10	134	115	130
Romania	0	0	17	39.2
Russia	79.3	327.6	390	589
Slovenia	0	0	20	0
Spain	640.8	267.7	202.2	281.1
Tunisia	551	357	408.5	95
Turkey	434	299.2	237.5	356
Ukraine	101.5	667.5	733	334.7
United Kingdom	36	24	24	0

Table 3. Soybean seed (HS code: 1201100000) for planting imports into EPPO countries from the USA from 2015-2018. The data for 2018 is from Jan-Nov. Figures detail in metric tonnes per year.

Country	2015	2016	2017	2018
Austria	0	2.8	268.8	232
Finland	5.3	0	0	0
France	0	13.2	183.5	196.4
Germany	435.4	450.9	20.7	15.6
Israel	0	0	14	0
Italy	11261.5	12476.4	12868.4	10109.1
Malta	0	0	5.8	0
Netherlands	10.6	0	9.7	155
Poland	29.2	0	0	0
Portugal	49.1	0	0	0
Romania	1269.4	6572.5	1761.3	161.5
Spain	0	0	0	37
Switzerland	0	89	110.3	0
Ukraine	40	0	0	0
United Kingdom	0	41.9	11.7	15.8